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CLINICAL AND SUBCLINICAL IRON-DEFICIENCY AMONG INNER-CITY ADOLESCENT GIRLS

Eric Donald Wells

1982

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Eric D. Well
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May 14, 1982
Date

CLINICAL AND SUBCLINICAL IRON-DEFICIENCY AMONG INNER-CITY ADOLESCENT GIRLS

Eric Donald Wells

A Thesis Submitted to the Yale University School of Medicine
in Partial Fulfillment of the Requirements for the Degree of
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ABSTRACT

The iron status of 76 inner-city adolescent girls aged 12-18 years attending Yale-New Haven Hospital Adolescent Clinic and the Hill Health Center was investigated employing laboratory tests, chart review, and a questionnaire. All subjects were free of inflammatory and chronic disease. 68% of the subjects were black, 28% Hispanic, and 4% white. Six were premenarchal and 5 were utilizing iron supplements. Two alternative definitions of iron-deficiency were presented: Criterion 1 (ID_1) was a serum ferritin <18 ng/ml and/or a zinc protoporphyrin ≥ 30 μg/dl blood, while criterion 2 (ID_2) was a serum ferritin <12 ng/ml and/or a zinc protoporphyrin ≥ 30 μg/dl. 25% and 33% of the 76 subjects met ID_2 and ID_1 respectively. 26% and 35% of the postmenarcheal girls who were not using iron supplements met ID_2 and ID_1. 11% of 76 and 12% of 65 subjects had anemia, defined as a hemoglobin <11.5 g/dl, with associated iron-deficiency. Iron-deficiency was found in all hemoglobin ranges (10.0-11.4, 11.5-12.4, 12.4-13.4, and ≥ 13.5 g/dl) employing either definition. The results of this study were compared to past studies of similar design. Over half of the patients with iron-deficiency were not identified using the traditional tests of hemoglobin and mean corpuscular volume, confronting clinicians with difficult decisions regarding the use of iron supplements and further laboratory tests.

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DEDICATION

Tina, Edwina, José, Brian

and

Frances

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INTRODUCTION

The most common cause of anemia during adolescence is iron-deficiency (1). Iron-deficiency anemia (IDA) is particularly common among adolescent girls of low socio-economic status (2,3,4). Poor diet and increased iron requirements due to puberty and menarche are thought to be etiologic factors producing increased susceptibility of teenage females to IDA. Etiologic factors common to all age groups and both sexes include blood loss due to anatomic gut lesions, gastritis, parasites, lung disease, epistaxis, kidney disease, and trauma, as well as impaired intestinal absorption due to diarrhea, inflammatory bowel disease, and malabsorption syndrome.

The production of hemoglobin, and ultimately the vascular transport of oxygen, are dependent upon the supply of iron. Many other proteins and enzymes also contain iron, or depend upon the presence of iron for their synthesis. It is well known that the decrease in oxygen transport during severe IDA is responsible for various clinical signs and symptoms. Iron-deficiency is also thought to result in pathophysiologic mechanisms, and subsequent clinical symptoms, due to a decrease in other iron-dependent proteins. The extent and importance of morbidity in the various organ systems containing these proteins are controversial. The degree of iron-deficiency necessary to produce physiologically relevant tissue changes is unknown. The importance of subclinical iron-deficiency, or iron-deficiency without anemia (sideropenia), is a current topic of debate (5).

There is no single laboratory test which can determine the presence and extent of iron-deficiency. Several tests, such as serum

ferritin (SF), free erythrocyte protoporphyrin (FEP), transferrin saturation (SAT), serum iron, mean corpuscular volume (MCV), hematocrit (Hct), and hemoglobin (Hgb), are available as tools in the diagnosis of iron-deficiency.

There are few studies in which the incidence of IDA has been investigated among inner-city adolescent girls. In only one study has the incidence of iron-deficiency independent of anemia been studied in a large population (6). There have not been any studies of sideropenia and IDA in a socioeconomically and/or racially homogeneous population.

IRON BIOCHEMISTRY

Iron is associated with various proteins in all tissues of the body. Iron is present in heme containing proteins, non-heme iron-containing enzymes, and as an enzyme co-factor(7).

In heme proteins, iron is bound to a protoporphyrin prosthetic group to yield heme. Heme proteins include hemoglobin, myoglobin, catalase, peroxidase, and the cytochromes. Hemoglobin is the body's oxygen transport protein, while myoglobin stores oxygen in muscle. Catalase and peroxidase are involved in peroxide metabolism. Cytochromes a, a_3, b, c, c_1, and oxidase are involved in electron transport in the mitochondrion. Cytochromes P450 and b_5 are located in the endoplasmic reticulum and participate in the degradation of drugs and endogenous poisons.

None-heme enzymes include the iron-sulfur proteins and the metalloflavoproteins. Examples are NADH dehydrogenase, succinic dehydrogenase, and xanthine oxidase.

Iron or heme is required as a cofactor by aconitase and tryptophan pyrrolase. Other enzymes which are iron dependent but in which the relationship is uncertain include ribonucleotide reductase and α-glycerophosphate dehydrogenase. Iron may be necessary for synthesis of these enzymes or as a co-factor.

Iron-deficiency has been shown to decrease concentrations of many iron containing compounds. The extent of iron-deficiency and the tissue involved appear to be important variables in concentrationreduction. The amount of iron-deficiency necessary to produce physiologically relevant reductions in iron containing proteins is controversial and yet to be determined.

CLINICAL SIGNS AND SYMPTOMS OF IRON-DEFICIENCY

Dietary disorders in iron-deficiency include anorexia and various forms of pica (8), including geophagia and pagophagia (9). Iron-deficient children have been shown to be significantly underweight when compared to normal children (10). Steatorrhea may be a symptom of IDA (11). Atrophic glossitis, atrophic gastritis, dysphagia, and esophageal webs are seen in adults with IDA, but are uncommon in children (8).

Central nervous system symptoms include fatigue, irritability, and lack of concentrating ability (8). Papilledema may be present in severe IDA. Weakness may be due to a muscular and/or a central nervous system basis.

Cardiovascular symptoms in severe anemia are pallor, palpitation, and dyspnea (12). Cardiac enlargement and murmurs may occur (8).

Skeletal changes may be apparent on roentgenpgraphs of the skull and metacarpal bones (8).

Splenomegally and hepatomegally may occur (12).

PATHOPHYSIOLOGY AND MORBIDITY IN IRON-DEFICIENCY

Cardiovascular and Musculoskeletal Systems

A decrease in Hgb concentration results in a decrease in the oxygen-carrying capacity of the blood (8). At very low Hgb concentrations (<7 g/dl), an increase in cardiac output and heart rate occurs at rest. These changes are seen in severe anemias of any etiology.

In IDA, peak exercise heart rates were significantly increased compared to controls (13). 15% more oxygen was delivered per pulse in iron replete than in iron-deficient individuals. However, there was no difference in oxygen consumption between the two groups. This could be explained by the increase in heart rate without significant change in stroke volume which is seen in exercising individuals with IDA (8). Gardner et al. (13) also found that the iron replete, non-anemic group had lower blood lactate levels than the anemic group, both in exercise and at rest.

Studies such as Gardner's have traditionally been used to support the concept that impaired maximal physical capacity in IDA is primarily due to decreased oxygen-carrying capacity of the blood (8). The other factor which may significantly contribute is tissue changes in skeletal muscle. Gardner's study suggests that tissue changes have occured, since lactate levels are constantly elevated in anemic subjects versus controls in the face of equal oxygen consumption.

Theoretically, one should be able to assess the relative contributions by doing two studies. In one, iron-deficient, anemic individuals would be transfused to Hgb levels equal to that of controls prior to exercise testing. Impaired work capacity in the iron-deficient group should be due to tissue changes. In the second study, iron replete individuals would have whole blood removed and replaced with plasma over

a short period of time. Exercise capacity would be compared to iron-replete controls with normal Hgb, differences being due to oxygen-carrying capacity of the blood.

Finch et al.(14) performed the first theoretical study in rats. Iron-deficient rats were significantly impaired in their running ability when compared to controls. After 4 days of iron therapy, the impairment was corrected. It was shown that concentrations of myoglobin, cytochromes a, a_3, b, and c, and rates of oxidative phosphorylation with pyrurate-malate, succinate, and α-glycophosphate as substrates were reduced in mitochondria from skeletal muscle of iron-deficient rats. The only activity which was totally reversed in the 4 days of iron therapy was that utilizing α-glycerophosphate. Alpha-glycerophosphate is a substrate for α-glycerophosphate dehydrogenase, which is necessary for the glycerol phosphate shuttle between the cytosol and mitochondria (15). It is thought to be an iron containing protein (14). It has been shown by Dallman and Schwartz (16) that the cytochrome c and myoglobin of rat skeletal muscle can not be restored after iron deficiency until 40 days of iron therapy. Finch et al. concluded that a decrease in α-glycerophosphate dehydrogenase activity was probably responsible for the impaired performance.

A second study (17) showed that iron-deficient rats had increased blood lactate levels after exercise when compared to controls. It was also shown that lactate infusion into normal animals interfered with work performance independently of pH. It was concluded that decreased levels of α-glycerophasphate dehydrogenase (oxidase) in iron deficiency impairs glycolysis, resulting in excess lactate formation, which in turn inhibits physical activity. Glycolysis would be impaired by the decreased ability to regenerate NAD from NADH in the cytoplasm.

Charlton et al. (18) studied cardiac frequency and minute ventilation over a long period of time on human volunteers made iron-deficient by repeated venesection. After being maintained in an anemic state for 6 to 8 weeks, the subjects were made iron replete by oral iron supplementation. The hemoglobin was kept at a low level by continued venesection. In one subject, they were able to show a significant change in cardiac frequency and minute ventilation during work on a treadmill (for 10 minutes) before and after iron repletion. The Hgb values before and after iron therapy were not significantly different. No SF, SAT, or FEP data were collected to show that the subject was truly iron replete. The data did appear to suggest, however, that tissue changes in iron-deficiency significantly impair work performance in humans.

The authors did not specifically report cardiac frequency or hemoglobin in these subjects prior to the study. It could be inferred from data provided in the report that the subject discussed earlier had a cardiac frequency of approximately 105/min. at one treadmill speed, and approximately 135/min. at a second, greater speed, prior to venesection. While iron replete, with a hemoglobin of 10.7 g/100 ml, the subject had cardiac frequencies of approximately 120 and 153 respectively. In that subject, the significance of these differences was apparently similar to that when he had a constant Hgb of 10.7 g/dl, while iron-deficient and replete. Tissue and hemoglobin changes were apparently of approximately equal significance in exercise impairment in this study, although it should be repeated in a more rigorous way.

Rothman et al. (19) investigated the effects of iron-deficiency on fracture-healing in rats. Chronic iron-deficiency anemia was found to greatly prolong non-union and to adversely affect fracture healing as determined by tensile strength and histological evaluation.

Gastrointestinal Tract

Dagg et al. (20) studied cytochrome oxidase activity in the buccal epithelium of 21 anemic, 16 sideropenic, and 8 normal subjects. Fifteen of the anemic patients, 7 with sideropenia, and none of the normals had markedly reduced to absent cytochrome oxidase activity. Cytochrome oxidase activity could not be correlated with the oral changes of atrophic glossitis or cheilosis.

Ghosh et al. (21) studied the basal and maximal gastric acid output in 18 children aged 6-13 years with severe IDA (Hgb$<$6.0 g/dl, hypochromic microcytic cells, decreased bone marrow hemosiderin). Mean acidity was significantly reduced when compared with 10 controls (Hgb$>$13.0). Naiman et al. (11) found 6 of 14 children with IDA to have reduced or absent gastric acidity which did not respond to histamine stimulation. 5 of 6 patients showed normal acidity after iron therapy.

Intestinal malabsorption and blood loss in iron-deficiency has been investigated by several laboratories. Yeomans and St. John (22) found decreased vitamin B_{12} absorption in iron-deficient rats. Bauerjee et al. (23) showed that decreased levels of serum and total folate may be present in IDA.

Kimber and Weintraub (24) studied the absorption of both heme and inorganic iron in newly-weaned dogs placed on an iron-deficient diet. Initially, iron absorption was increased over controls. With increasing severity of iron-deficiency, absorption was decreased below that of controls.

In mild to moderate IDA in children, iron absorption from the diet is usually increased (8, 25). Absorption of iron is impaired in some children with IDA (25). Heme iron absorption from the diet is impaired in severe IDA (24). In both inorganic and heme iron impairment, absorption returns to normal with iron repletion.

Guha _et al_. (12) investigated small bowel function in children aged 4-12 years with severe IDA. Occult blood in the stool, xylose absorption, and stool fat excretion were studied before and after iron therapy. Six of 16 with occult blood in their stools, 12 of 17 with an abnormal xylose test, and 14 of 18 with increased stool fat before therapy were all found to be normal after correction of anemia. Since 23 of the 26 children involved in the study had normal serum albumins, the authors concluded that the intestinal dysfunction could not be due to protein deficiency. Naiman _et al_.(11) studied 14 children under 3 years of age with iron-deficiency anemia. Six of 14 showed impaired xylose absorption, 5 of 14 had decreased serum carotene, 10 of 14 had abnormal vitamin A tolerance curves, 4 of 14 showed increased stool fat, and 4 of 14 had positive stool guiac tests. Most of these tests returned to normal after iron therapy had corrected the anemia. Eight patients with anemia of other etiologies were also studied. Vitamin A tolerance and serum carotene were abnormal in these patients. Lanzkowsky _et al_. (27) showed that 6 of 10 children from 8 to 30 months of age with severe iron-deficiency had impaired lactose tolerance. Ghosh _et al_. (2) were unable to show any significant difference in fecal fat excretion or xylose absorption between controls and children with iron-deficiency anemia.

It is apparent that there is a malabsorption syndrome associated with iron-deficiency anemia involving vitamin B_{12}, lactose, iron, and probably fats. Some biochemical correlates in the intestinal epithelium have been studied (see below). Few studies have been done which show that these abnormalities are due specifically to iron-deficiency, and not anemia. Whether iron-deficiency anemia is associated with intestinal blood loss is less certain. The patients in Naiman's study were ingesting

cow's milk on a regular basis. Intake of milk was decreased when iron therapy was begun. Intestinal blood loss in iron-deficient infants is commonly due to whole cow's milk induced bleeding (26).

Various enzymes in the intestinal epithelium have been studied in iron-deficiency anemia. Lanzkowsky _et al_. (27) found that 6 of 10 severely iron-deficient children had abnormal lactose tolerance. Intestinal biopsies were obtained from 5 of these 6. Reduced disaccharidase activities (lactase, sucrase, and maltase) were demonstrated in all 5 samples. Kimber and Weintrab (24) found decreased levels of lactase in the internal mucosa of iron-deficient dogs. Intestinal disaccharidases are not thought to require iron for activity. Lanzkowsky (27) reports that iron-deficiency in rats yields an alteration in the entire protein file of polyacrylamide gel scans. He concludes that iron-deficiency alters the protein synthesis mechanism in a quantitative, not qualitative, way.

Dallman and Schwartz (16) showed that the cytochrome C of the intestinal mucosa could be reduced 50% by feeding rats an iron-deficient diet for 56 days. Correction with oral ferrous sulfate yielded normal cytochrome C values in 2 days. Cytochrome oxidase levels were reduced with iron-deficiency, and also returned to normal with 2 days of iron therapy. Dallman _et al_. (28) studied 5 infants with severe iron-deficiency undergoing intramuscular iron-dextran therapy. In one of those patients, a biopsy 24 hours after initiation of therapy showed cells at the base of the villi to be darkly stained for cytochrome-oxidase activity, while those at the tips of the villi were more lightly stained. After 72 hours, all cells were darkly stained. Hoensch _et al_. (29) studied the drug-metabolizing (microsomal) enzymes of the intestinal epithelium

in iron-deficient rats. Cytochrome P450 and benzpyrene hydroxylase showed significantly decreased activity. Activity was completely restored after 24 hours by oral iron supplementation, but not by parenteral iron administration. Lanzkowsky _et al_.(27) reported decreased succinic dehydrogenase activity in 4 of 5 infants with severe iron-deficiency on whom intestinal biopsies were performed.

In both the rat and man, the lining of the small intestine is replaced every two days, while heme protein repair in the intestinal mucosa also takes two days. Dallman _et al_. and Hoensch _et al_. conclude that utilization of iron to produce iron containing enzymes is limited to newly-developing intestinal epithelial cells. This idea is supported by the fact that rates of heme protein repair in red cells and muscle parallel known variations in the cell renewal rates of these tissues in the rat (28). Hoensch _et al_. further postulate that only iron from the intestinal lumen can be utilized.

Morphological changes in the intestinal mucosa have also been reported in iron-deficiency. Guha _et al_. (12) reported abnormalities in 11 of 15 patients, including cellular infiltration of the lamina propria, fusion, blunting, and atrophy of villi, reduction in the number of villi, and atypical villous epithelial cells. Naiman _et al_. (11) reported similar findings in 7 of 11 patients. Both groups reported improvement after iron therapy. Ghosh _et al_. (21) were unable to show significant changes in jejunal biopsy specimens from 20 iron-deficient subjects. Five infants with severe iron-deficiency anemia studied by Lanzkowsky (27) were shown to have normal intestinal mucosa by light and electron microscopy.

Central Nervous System

Biochemical Studies

Dallman et al. (30) investigated non-heme iron content in brains of young, iron-deficient rats. Between the 10th and 28th days of life, the rats received an iron-deficient diet. The concentration of non-heme brain iron was found to be 22% below controls after 28 days. After iron-deficient rats were treated with intra-muscular (IM) iron for 14 to 45 days, none-heme brain iron remained depressed at 19-29% below the mean concentration of controls. Brain ferritin also remained depressed at 33-42% below control means. The Hct, liver non-heme iron, and liver ferritin were severely depressed in iron-deficient rats, but promptly returned to control values after treatment.

Dallman and Schwartz (31) found brain cytochrome C concentrations to be resistant to significant decreases in adult rats made iron-deficient. Young rats were also resistant (32). Only when pregnant rats were made iron-deficient, followed by iron-deprivation of offspring, could brain cytochrome C be shown to be significantly lowered.

Monoamine oxidase (MAO), an enzyme involved in the degradation of the neurotransmitters dopamine, norepinephrine (NE), and serotonin, is thought to be iron dependent (33). Symes et al.(34) investigated liver MAO activity in iron-deficient rats. After 5 weeks of iron deprivation, liver MAO activity was 18-31% below that of controls. The mean total liver iron decreased 64%. Hgb decreased by a ratio similar to that of total liver iron.

Youdim et al. (35) studied human platelet MAO activity in patients with Hgb <10g/dl, MCV <76 fl, decreased serum iron, and decreased SAT. MAO activity was significantly reduced 37-52%, depending on the substrate used. MAO activity returned to normal in patients receiving oral iron

therapy if serum iron levels returned to normal.

Youdim and Green (36) studied several brain enzymes in rats after 5 and 9 weeks of iron deprivation. At 5 weeks, there was no decrease in brain MAO or aldehyde dehydrogenase activity. At 9 weeks, no decrease could be detected in brain tryptophan hydroxylase or succinate deydrogenase activity, although succinate dehydrogenase activity declined markedly in the peripheral tissues of the same animals. Brain MAO activity decreased a small but significant 15% after 9 weeks of iron deprivation. Serotonin and catecholamine precursors were administered to control and iron-deficient rats. No difference in dopamine or serotonin accumulation was noted between the two groups. Subsequent hyperactivity, though, was significantly decreased in iron-deficient rats. Youdim and Green concluded that a defect might be present in the post-synaptic response of the iron-deficient rats, since no pre-synaptic defect could be detected. Whether the hyperactivity was a CNS-mediated behavioral effect or one caused by peripheral effects of iron-deficiency is difficult to determine. The Hgb was reduced to less than one half of that of controls, which may have impaired oxygen delivery to peripheral muscle. Decreased levels of skeletal muscle enzymes with resultant increases in lactate levels detrimental to muscle performance have been demonstrated in iron-deficient rats (14,17).

Voorhess _et_ _al_.(37) described abnormally high urinary excretion of NE in 11 children with Hgb <10.2 g/dl and SAT <15%. After 1 week of iron therapy the NE excretion decreased significantly and returned to a normal level. All patients corrected their anemia within 6 weeks. Four of 5 anemic, "iron replete" children showed no significant decrease in NE excretion after transfusion. The authors speculated that increased NE excretion in iron-deficiency could be related to decreased brain MAO degredation

of NE. It has been previously noted that symptoms ascribed to iron-deficiency, such as irritability and anorexia, are alleviated after a brief course of iron-therapy. (38) Voorhess et al. noted that these symptoms disappear during a short period similar to that of the decrease in urinary NE excretion, long before significant increase in Hgb.

Aldehyde oxidase is an enzyme in the pathway of serotonin degradation, Mackler et al. (39) found significantly decreased aldehyde oxidase activity in 8 week old rats after 1 month of iron-deprivation. The authors also found increased concentrations of serotonin and 5-hydroxyindole compounds. Both findings (enzyme activity and chemical concentrations) were in direct contradiction to the results of Youdim and Green (30) discussed above. One possible reason for the discrepancy could be the age of the rats used in the respective laboratories. Youdim and Green do not mention the age of their animals, while Mackler et al. used relatively young rats. As discussed previously, older rats are more resistant to changes in brain cytochrome C induced by iron-deprivation than are younger rats.

Behavioral Studies

Cantwell (40) studied 61 6-7 year old children from similar socioeconomic groups for long-term sequelae of anemia in infancy. Thirty-two patients had hemoglobins between 6.1 and 9.5 g/dl between 6-18 months of age. Twenty-nine controls who had iron-dextran injections as infants did not become anemic as infants. The anemic children had a higher incidence of "soft" neurological signs, were less attentive, more hyperactive, and scored lower on Binet IQ tests (92 vs. 98) than the controls. No statistical information was given.

Oski and Honig (41) studied 24 infants aged 9-26 months with Hgb <10.5 g/dl, MCV ≤ 73 fl, and SAT $\leq 12\%$. One half the patients received iron-dextran injections and the other half received placebo injections (controls). All infants were tested on the Bayley Development Scale before and after injection. The control group improved 6.1 points on the Mental Development Index (not statistically significant) while the treatment group improved 13.6 points (statistically significant) in a mean time of 6.8 days. The test was well-controlled socioeconomically.

Sulzer *et al*. (42) studied 4 and 5 year old children with Hgb <10.5 g/dl in a Head-Start program. The anemic group consistently scored significantly lower on two IQ tests as well as in an associative learning test when compared to controls.

Webb and Oski (43) investigated scholastic achievement in 92 anemic(Hgb 10.1-11.4 g/dl) and 101 non-anemic (Hgb 14.0-14.9 g/dl) 12-14 year old children. Anemic females consistently scored lower than controls at ages 12, 13, and 14 years. Anemic males scored similarly to controls at age 12 years, but progressively declined to significantly lower scores at ages 13 and 14 years. Conduct disturbances in anemic males were reported to be higher than in controls by school teachers (44). A signifcantly longer latency in reporting visualization of an after-image was found in anemic (4.08 seconds) than in non-anemic (1.81 seconds) subjects.

The behavioral studies reviewed above have several methodological flaws (33), including lack of statistical analysis, inadequate data, and lack of documented socioeconomic controls. Webb and Oski mention that their findings may be due to poor general (versus iron) nutrition. The Bayley Tests are difficult to administer objectively and consistently.

Although the preceding studies suggest that IDA is responsible for behavioral deficits, they do not differentiate between the effects of iron-deficiency and anemia. Two recent studies attempt to investigate the effects of isolated iron-deficiency upon infant behavior.

Deinard et al. (45) studied 212 infants aged 11-13 months. All infants were described as non-anemic (Hct $\geq$34%). The patients were divided into 3 groups, depending on SF values: SF $\leq$9 ng/ml, SF 10-19 ng/ml, and SF $\geq$20 ng/ml. No statistically significant differences could be demonstrated among the 3 groups in cognitive or behavioral function using the Bayley tests.

There are several flaws in the design of Deinard's study. Since the low-normal limit of SF may be lower among young children than among adults (46), the mean value of 8.85 ± 1.58 hg/ml for the first group is not an impressive standard for "severe iron-depletion," as defined in the study. Decreased storage iron, as revealed by SF, is the first stage of iron deficiency (47). Decreased storage iron does not necessarily limit the iron which is incorporated into the various iron dependent proteins of the body (including the brain). A much better criterion for judging such limitation is an elevated FEP. If the FEP is increased the iron supply is limiting the formation of heme. Formation of other heme-containing proteins, as well as non-heme iron-dependent proteins, is also likely to be limited. Hct is used as a criterion for anemia, but is thought to sometimes yield false-high values (47). The study is retrospective, an argument often used against past behavioral studies with positive results (33).

Oski et al.(48) studied 33 infants aged 9-13 months with the Bayley Mental Development Index (MDI). Ten controls had Hgb $\geq$11.0 g/dl,

SF >12 ng/ml, FEP <30 μg/dl, and MCV >70 fl. Eight "iron-depleted" infants had Hgb >11.0 g/dl, SF <12 ng/dl, FEP <30 μg/dl, and MCV >70 fl. Fifteen "iron-deficient" infants had Hgb >11.0 g/dl, SF <12 ng/dl, and FEP ≥30 μg/dl. All subjects were administered the MDI, treated with IM iron, and then retested one week later. The mean increases were 7.2 for the normals, 3.7 for the iron depleted infants, and 21 for the iron-deficient infants. Only the iron-deficient infants had a statistically significant increase.

The effects of iron-deficiency on the nervous system remain controversial. Brain enzymes appear to be resistant to depletion in rats, although reductions in brain enzyme activities may occur in young, developing rats. The effects of iron-deficiency on brain enzymes in man are unknown. If parallel to results in rats, iron-deficiency may have important CNS consequences in infants, as suggested by Cantwell and Oski.

Behavioral studies in iron-deficiency are plagued by poor environmental controls and subjective data-collecting methods. The irritability, anorexia, and dietary disorders commonly noted in iron-deficiency suggest that there are real effects on the nervous system. The association with intellectual impairment, acute behavioral effects, and long-term sequelae are yet to be satisfactorily established.

Physical Growth and Development

Judisch _et al_. (10) studied 88 infants with iron-deficiency anemia (Hgb < 9 g/dl). All infants had low serum iron and decreased transferrin saturation or a good response to iron therapy. Weight was measured before and after iron therapy, which was provided until 2 months after the Hgb returned to normal. Pre-therapy, 15% of the patients were below the 3rd percentile in weight and 60% were below the 50th percentile.

Post-therapy, 2% were below the 3rd percentile and 45% were below the 50th percentile. No statistical analysis was presented.

Garn and Smith (49) studied data from the 15,000 1-17 year old children of the Ten-State Nutrition Survey of 1968-70. The children with the top 15% of Hgb values were compared to those with the bottom 15%. The top 15% were 3 cm. taller at 6 years of age and 3 kg heavier until adolescence than the low 15%. Height differences increased after 6 years of age and weight differences increased during adolescence. These differences were true within white and black racial groups. No statistical information was given.

Immunology

Andelman and Sered (50) studied 1,048 infants of low socioeconomic class, 603 of whom received iron-fortified formula. The group receiving the supplemental iron had significantly higher Hgb concentrations, as well as a significantly lower incidence of respiratory infections. McKay (51) and Salmi _et al_. (52) found similar results in earlier studies.

Conolly and Golovsky (53) prospectively studied the relationship between wound infection and anemia in surgical patients. Twenty percent of patients with infections had Hgb $<$ 10 g/dl, while only 7% of patients without infection had Hgb $<$ 10 g/dl.

Cummins _et al_. (54) found that rejection of the parasite _Nippostrongylus brasiliensis_ was impaired in rats deficient in both iron and protein. Iron-deficiency alone was not studied.

Fletcher _et al_.(55) found that the saliva of patients with atropic glossitis and angular chilosis associated with IDA contained more Candida than did saliva of controls. The saliva of the IDA patients

also supported the growth of Candida more readily. Higgs and Wells (56) found that 23 of 31 patients with chronic muco-cutaneous candidiasis were iron-deficient. Fifteen of the 23 were sideropenic (decreased serum iron, reduced or absent bone marrow iron, and Hgb ≥12.0) and 8 of 23 suffered from IDA. Nine of 11 patients treated with iron showed significant clinical improvement.

Although the studies noted above have suggested an increase in infections in IDA, and possibly in siderpenia as well, other studies have failed to show an increase (57). Studies at the cellular and biochemical levels have usually supported the idea that immune system function is compromised in iron-deficiency.

Several laboratories have studied lymphocyte transformation following phytohemagglutinin stimulation in iron-deficient subjects. Joynson *et al*. (58) and Fletcher *et al*. (55) showed impaired transformation in subjects of various ages with IDA. Srikantia *et al*. (59) and MacDougall *et al*. (60) found impaired transformation in children with IDA. Kulapongs *et al*.(61) were unable to show any impairment in 8 children with IDA, 7 of whom had hookworm infestation.

Lymphocyte bactericidal function was found to be reduced by several investigators (59,60,62), but normal in one study (61). Joynson *et al*. (58) found a decrease in migration inhibition factor production in IDA. MacDougall *et al*.(60) showed increased neutrophil chemotactic activity. Chandra (62) and MacDougall *et al*. studied reduction of nitroblue tetrazolium by neutrophils in subjects with IDA. Chandra found reduced activity while Macdougall *et al*. were unable to show an abnormality. Baggs and Miller (63,64) found a direct relationship between the amount of myeloperoxidase and dietary iron in rats' small intestine and cecum.

Nalder *et al*. (65) found a direct relationship between the reduction in dietary iron and the reduction of antibody production in rats. Macdougall *et al*.(60) were unable to show a decrease in serum immunoglobulin concentration or salivary IgA in children with IDA.

Although the relationship between immunocompetence and iron-deficiency remains a matter of debate, there is mounting evidence that there is a positive correlation. One can postulate that decreased iron limits the production of iron-dependent enzymes, such as myeloperoxidase. Manifestations of changes at the biochemical level would be manifested by cellular and humoral changes, resulting in an increased incidence and severity of infection.

It is interesting to note that addition of iron to serum *in vitro* as well as intravenous and intramuscular addition of iron *in vivo* have been shown to increase the growth of various bacteria in serum (66,67). Weinberg's hypothesis of "nutritional immunity"(68) postulates that unsaturated transferrin is protective against bacterial infection and states that iron therapy may reduce resistance by saturating transferrin. However, Pearson *et al*. (69) were unable to show any difference in rates of growth of *E. coli* in sera of individuals with SAT from 6-100%. The additional iron of other studies may have been added at a rate and in quantities that left much of it loosely bound to other serum proteins. Such a situation should not occur with oral iron replacement therapy.

Red Blood Cells and Bone Marrow

In IDA there is increased destruction of erythrocyte precursors, or ineffective erythropoiesis, within the bone marrow (8). DNA and RNA synthesis is impaired, and heme production is reduced in normoblasts.

There is thought to be a defect in vitamin B_{12} metabolism which leads to hypersegmentation of neutrophils (70).

Red cell survival is reduced to less than one half of normal due to increased hemolysis (8). This destruction occurs only in the presence of a functional spleen.

Synthesis of the α and β chain subunits of hemoglobin is reduced in IDA (8). The α:β-chain ratio is also reduced.

Macdougall found decreased glutathione peroxidase and catalase activities in 15 children with IDA (71). The iron-deficient red cells were more susceptible to hydrogen peroxide hemolysis than controls. It is thought that the loss of the peroxide detoxifying enzymes may lead to oxidative damage to red cell membranes (8). Red cells become less deformable after such damage and are more easily trapped by the spleen.

2,3-DPG, glutamic oxaloacetic transaminase, NADH, and methemoglobin reductase activity in red cells are increased in IDA (8).

It is legitimate to conclude that IDA leads to pathological conditions in several organ systems. Cardiovascular decompensation, impaired muscle activity, biochemical, functional, and morphological gastrointestinal effects, impaired physical development, cellular immune dysfunction, and biochemical and morphologic erythrocyte alterations are almost definite sequelae of IDA. An increased incidence of infections, and behavioral and intellectual impairment must also be considered possible results.

Little research has been conducted on the physiologic effects of sideropenia. Studies have been mentioned reporting decreased cytochrome oxidase activity in buccal mucosa, behavioral impairment of infants, and increased growth of Candida in saliva in sideropenia. Conclusions regarding the relationship between subclinical iron-deficiency and morbidity must await further investigations.

LABORATORY VALUES IN THE DIAGNOSIS OF IRON-DEFICIENCY

Although the diagnosis of iron-deficiency depends upon laboratory analyses, the determination of a significantly iron depleted state is not straightforward. Theoretically, iron-deficiency progresses through several stages before the development of anemia (47). In the first stage, there are decreased or absent stores of iron in the liver and bone marrow, reflected by a decreased concentration of SF. In stage two there is a decrease of plasma transport iron, measured by the SAT. In stage three the transport iron decreases to an extent that limits the production of heme, and FEP levels begin to rise. In the fourth stage, there is a detectable decrease in the concentration of Hgb in whole blood due to the decrease in heme production. The decrease in Hgb level is often accompanied by changes in cellular morphology and cell numbers, reflected by decreased MCV, Hct, and mean corpuscular hemoglobin concentration.

In individuals, laboratory results often fail to conform to this pattern (47). Almost any combination of abnormal and normal laboratory values may occur. Some of the reasons will be discussed below.

Serum Ferritin

Ferritin is an intracellular iron storage protein found principally in the cytoplasm of reticuloendothelial and liver cells (8), but also in other tissues, including brain cells (30). The minute amounts of ferritin which are found in serum can be measured by radioimmunoassay. SF levels are thought to reflect the amount of iron stores in the body (72). Cook *et al.* (73) showed a correlation between SF and iron stores measured by radioiron absorption. Luxton *et al.* (74) and Mazza *et al.*(75) showed a high correlation between low SF and lack of marrow stainable iron.

Walters et al. (76) found a high correlation between SF concentration and storage iron measured by quantitative phlebotomy. Biopsied liver tissue has an iron content which can be correlated with SF (77).

Published low-normal limits (LNL) have varied between 7 and 18 ng/ml, depending upon the definition of LNL, age group, and sex. Siimes et al. (46) studied "normal" children between 6 months and 15 years of age with Hct within 95% confidence limits. They found a median SF concentration of 30 ng/ml, and defined the LNL as the 95% confidence limit of 7 ng/ml. Six children with "latent iron-deficiency," defined as SAT <16% but without anemia, had a median SF of 10.6 ng/ml with a range of 4.5-51 ng/ml. Deinard et al. (45) cited Siimes et al. in defining a SF ≤ 9 ng/ml as "severely iron-depleted" and a SF from 10-19 ng/ml as "mildly iron depleted." In the original study, many of the "normal" children may have been iron-deficient. The Hgb can be low with a normal Hct (47). No studies, such as SAT or FEP, were reported to rule out iron-deficiency independent of anemia.

An often cited study by Cook et al. (73) investigated 326 adults from 20-50 years of age with FEP <95 µg/dl, SAT >20%, and Hgb >12 g/dl in females and >13 g/dl in males. The LNL was defined as the 95% confidence limit, yielding a value of 9 ng/ml in females and 12 ng/ml for the entire sample.

Lipschitz et al. (77) found a SF concentration range of 1-14 ng/ml in 32 patients with "uncomplicated iron-deficiency" (SAT < 16%, TIBC > 400 µg/dl). In 12 other patients with no known systemic disease and absent bone marrow hemosiderin, the SF mean was 6 ng/ml with a range of 1-37 ng/ml. In 8 patients with "diminished" bone marrow hemosiderin,

the range was 21-163 ng/ml. In no individual was a value of 20 ng/ml or less associated with the presence of stainable iron in the marrow.

Luxton et al.(74) studied 60 women aged 20-49 years and 60 women aged 50-79 years. Elimination of patients with evidence of chronic disease yielded 95% confidence limits of 4-144 ng SF/ml and 9-200 ng/ml respectively. A separate group of 248 women undergoing bone marrow aspirations were evaluated for bone marrow hemosiderin and SF. The absence of stainable iron in the bone marrow correlated with SF < 18 ng/ml. 30% of the original 60 women aged 20-49 had no bone marrow iron stores using this criterion (comparable to 24% of young college women without iron stores in biopsied bone marrow in a study by Scott and Pritchard (78)). Using the criterion of Luxton et al., Mazza et al.(75) found no false-negative values in 19 patients lacking marrow stainable iron who were without systemic disease. In 67 patients with various chronic diseases and positive for stainable iron, there were 3 false-positive values. Cook et al. (6) studied various laboratory parameters of iron-deficiency in 1564 subjects. 17.5% had FEP >100 μg/dl, 14.2% had SAT < 15%, 13.5% had SF <12 ng/ml, and 8.3% had Hgb <3rd percentile. Since SF is thought to be the first laboratory parameter to become abnormal in iron-deficiency, one would expect it to have the greatest incidence of low values. A LNL of 12 ng/ml may therefore be too low an estimate.

Various diseases have been associated with false-negative SF concentrations in iron-deficiency. Koerper et al. (79) studied patients with juvenile rheumatoid arthritis. Many patients with normal or high SF responded to iron therapy with increases in Hgb and MCV. None had been receiving iron supplements in the previous 6 months.

Elin _et al_. (80) injected volunteers with bacterial endotoxin, etiocholanolone, and a control substance to induce fever. SF levels increased significantly for up to 10 days after injection with the pyrogenic compounds.

Siimes _et al_. (46) found increased median concentrations of SF in children with chronic hemolytic anemias, upper and lower respiratory tract infections, fever of unknown origin, gastroenteritis, and acute lymphoblastic leukemia. Lipschitz _et al_.(77) found values above the upper limit of normal (300 ng/ml cited from Cook _et al_. (73)) in inflammatory disease, liver disease, and diseases with increased RBC turnover.

Increased SF levels in hemolytic disease are due to iron overload. Increased levels in other diseases may be related to the movement of iron into storage compartments, which is thought to occur in infectious diseases(68).

SF values increase after only a few days of iron therapy (47). Other indices of iron-deficiency, such as FEP, MCV, and Hgb, change much more slowly. A seemingly anomalous normal ferritin may therefore be noted with a high FEP, low Hgb, and/or low MCV in an iron-deficient individual.

Free Erythrocyte Protoporphyrin

The limitation of iron available for incorporation into heme results in the accumulation of unused protoporphyrin in normoblasts (8). Dagg _et al_.(81) studied concentrations of FEP in subjects with iron-deficiency anemia (SAT <16%, Hgb <12 g/dl, absence of bone marrow hemosiderin), sideropenia (SAT <16%, Hgb 12-13 g/dl, absence of marrow hemosiderin), and in controls (SAT >16%, Hgb >13 g/dl, presence of normal marrow iron). The mean FEP in IDA (160 ± 97 μg/dl) was significantly higher than that in sideropenia (93 ± 60 μg/dl), which in turn was significantly higher than

that in controls (15.5 ± 8 μg/dl). The accumulation of FEP was shown to be an intermediate stage in the development of IDA.

The FEP concentration returns to normal only after several months of sufficient iron intake (81). Because FEP exists intracellularly, the decrease in FEP is thought to depend upon the elimination of old cells which were formed during an iron-deficient state.

The porphyrin present in erythrocytes during iron-deficiency and plumbism has been shown to be chelated with zinc (82). The porphyrin present in erythropoietic protoporphria was not in the form of zinc protoporphyrin (ZPP). The optimum wave length of light for exciting ZPP fluorescence was 423 nm.

The FEP concentration has traditionally been measured in erythrocytes by the use of an acid-extraction method and a fluorometer (83, 84, 85). The excitation maximum of ZPP was utilized in the development of a hematofluorometer (86), a device measuring the ZPP concentration in whole blood. The machine was designed to analyze a thin film of blood on a disposable glass coverslip. The FEP concentration is reported in units of μg per dl packed erythrocytes (pRBC) when determined by the extraction method. The ZPP, as measured by hematofluorometer, is measured in μg per dl whole blood.

Treatment of blood with heparin, EDTA, or oxalate anticoagulants does not alter the results of the hematofluorometer (86). Blood may be refrigerated in the dark for one week without change in readings. Extensive hemolysis of RBC has little effect on results.

A universal high-normal limit (HNL) for FEP concentration has not yet been established to to poor intralaboratory correlation, even with the use of a common method (87). Langer *et al*. (83) defined 70 μg/dl

pRBC as the HNL after studying 20 "normal" men (MCV, SAT, serum iron, and Hct within normal limits) with a range of 16-67 μg/dl. McLaren et al. (88) found bone marrow hemosiderin to be absent in all patients with FEP >94 μg/dl pRBC. In a recent review article (89), the HNL is defined as 70 μg/dl pRBC, 30 μg/dl blood, or 3 μg/g Hgb. In a recent study, Oski et al. (48) used ≥30 μg/dl blood as the HNL. Cook et al. (6) used a HNL of 100 μg/dl pRBC.

FEP concentrations are thought to be increased in inflammation (47). Concentrations are increased in tuberculosis (83). FEP values are moderately to very high in plumbism (82, 90). With the use of a hematofluorometer, spuriously high results occur in the presence of bilirubin (91), with unoxygenated blood (86,91), and with decreasing Hct (86). In surveys comparing extraction and hematofluorometer methods, the latter method had a low bias(87, 92), although correlation between the methods was high.

Transferrin Saturation and Serum Iron

Transferrin is an iron-specific transport protein of the body's intravascular and extravascular spaces (8). It is synthesized primarily in the parenchymal cells of the liver. The SAT is calculated by dividing serum iron concentration by total binding capacity (TIBC) and multiplying by 100. TIBC and serum iron are measured spectrophotometrically.

The LNL of SAT in adults is 16% (47). Between 6 months of age and adolescence, the SAT LNL ranges from 7% to 10%. TIBC is usually in the vicinity of 200 μg/dl, but may increase to 400 μg/dl or more in iron-deficiency. Serum iron has a LNLfrom10-16 μg/dl.

Beginning at age 3 years, there are significant diurnal variations in serum iron concentrations (93). Because of the high variability of

SAT and serum iron with age and time of day, they are better employed as confirmatory tests of iron-deficiency than for initial determination.

Mean Corpuscular Volume (MCV)

A low MCV is most commonly associated with IDA, thalassemia minor, and plumbism. The MCV is measured directly by electron counter. The LNL for white non-indigent girls aged 12-13 years is 77 fl, and 78 fl for girls aged 14-18 years (95% confidence limits) (94).

The differentiation among the various etiologies of a low MCV must be accomplished through further laboratory analysis (95). Hgb A_2 >3.5% by quantitative Hgb electrophoresis or microcolumn chromatography establishes a diagnosis of β-thalassemia trait. Hgb A_2 >3.5% plus abnormal iron status tests usually indicates IDA. A high FEP must be further investigated for plumbism. A low MCV with Hgb A_2<3.5% and normal iron studies usually indicates α-thalassemia trait. Alpha-thalassemia may yield low Hgb levels independent of superimposed iron-deficiency. To confirm a diagnosis of α-thalassemia trait, family genetic studies or restriction endonuclease mapping (96) must be performed.

Alpha-thalassemia is most frequent among Mediterranean ethnic groups (95) and Oriental (96) and Black (97) racial groups. The form most often found in black Americans is usually of no clinical significance. 29.4% of black Americans have α-thalassemia genes, most in the "silent carrier" form, as elucidated by restriction endonuclease mapping (97).

Hematocrit

The Hct is determined directly by centrifugation or indirectly by calculation (MCV x RBC count). The Hct usually equals the Hgb value multiplied by three using mean values in studies of large populations. In anemic individuals, many falsely elevated values are believed to occur. For adolescent girls, LNLs of 34% (2) and 35% (98) have been used in population studies.

Hemoglobin

Anemia is usually defined as the decrease in hemoglobin concentration and/or total number of RBC below a LNL (8). Anemia develops from a variety of etiologies. Blood loss is an important cause. Decreased RBC production results from nutritional deficiencies (i.e., vitamin B_{12}, folate, copper, protein, vitamin C, riboflavine, pyridoxine, and iron), from bone marrow depression or failure, and from ineffective erythropoiesis. Increased RBC destruction is associated with hereditary diseases (e.g., hemoglobinopathies, thalassemias), immune disease, chemical and infectious agents, and physical trauma to the erythrocyte.

Hgb is measured spectrophotometrically after dilution into a solution which converts it into cyanmethemoglobin. No single LNL is applicable to all individuals (47). Normal and abnormal Hgb concentrations have an overlap of approximately 2 g/dl.

Garby _et_ _al_. (99) considered IDA to exist in individuals showing a significant response in Hct or Hgb following iron therapy. Almost all female subjects of a Swedish population with a Hct <33% showed a significant response, while only 50% of patients with a Hct = 36% responded. Subjects with a Hct as high as 41 showed a response. The total frequency of response was 17%. In this population, 15% had Hgb <11.5 g/dl and 30% had a Hgb <12.0 g/dl. Natvig _et_ _al_.(100) showed that iron therapy may result in an increase in Hgb value without an increase in Hct. Garby _et_ _al_. do not reveal the frequency of response of Hgb values. Whether or not an increase in Hgb or Hct values after iron-therapy occurs only in IDA or is physiologically relevant is unknown. If the stated definition of IDA is accepted, a Hgb LNL of 11.5 g/dl would have given a good approximation of the frequency of IDA in the population studied.

Natvig et al. (100) made Hgb determinations in apparently healthy Oslo school children. LNLs for girls, based upon 95% confidence limits, were suggested to be 11.6 g/dl from 10-13 years and 12.2 g/dl from 14-20 years. Based upon 95% confidence limits, Dallman and Siimes (94) established a LNL of 12.0 g/dl in non-indigent white girls aged 12-18 years.

Hillman and Smith (4) found high frequencies of anemia in Brooklyn using a LNL of 11.0 g/dl in all age groups. Katzman et al. (98) used a LNL of 11.5 g/dl for girls aged 12-15 years in a homogeneously black population. In order to validate the definition of anemia utilized, mean MCV values were determined for 3 groups. Group one had a Hgb range of 10.0-10.9, group 2 had a Hgb range of 11.0-11.4, and group three had a Hgb range of 14.0 to 14.9. Group three was assumed to contain individuals without iron-deficiency. The mean MCV of group three was significantly higher than those of groups one and two, which did not differ significantly from each other.

Dallman et al. (101) studied Hgb values of black and white children in San Francisco. The two racial groups were controlled for hemoglobinopathies, iron-deficiency, thalassemia minor, obesity, and socioeconomic status. Median Hgb concentrations in white subjects averaged 0.5 g/dl greater than those of black subjects over all ages. In girls aged 10-14 years, LNL of 12.0 g/dl for white subjects and 11.2 g/dl for black subjects were calculated using 95% confidence limits. Similar differences in mean values were described by Owen et al. (102). Garn et al. (103) found median Hgb concentrations in blacks to be 1 gm/dl less than in whites from the first through ninths decades of life when analyzing the 1968-70 Ten-State Nutrition Study. This study was less well controlled than the previous two. None of the studies were well controlled socioeconomically,

using only gross differences in per-capita income and parents' education as indices. No cultural variables, such as diet, were controlled.

Garn *et al*. (103) studied Hgb values in 39 black and 32 white infants from birth through 18 months of age. They were maintained on a supervised diet with iron in excess of recommended dietary allowances. There was a difference in hemoglobin levels of approximately 0.5 g/dl beginning about the fifth month of life, extending to the eighteenth month, when the study was terminated.

The Use of Multiple Laboratory Values in Population Studies

Dallman *et al*. (104) studied 1,128 infants aged 1 year in a U.S. military population. Laboratory values were assessed for ability to predict response to oral iron therapy. An increase in Hgb 1 g/dl was defined as a "physiologically relevant" response. It was determined that in infants with Hgb <11.5 g/dl (10th percentile), abnormal SAT, MCV, FEP, and SF were poor predictors of response, alone or in combination. Many false-positive (non-responders) were identified as iron-deficient. Any method of combining abnormal tests with low Hgb levels which increased the number of responders also increased the number of false-positives. If the criteria for iron-deficiency was Hgb <11.5 g/dl and at least 1 abnormal value among the other 4 tests, approximately 90% of the responders were identified. 55% of the subjects meeting the criteria did not respond. If Hgb was <11.5 g/dl and none of 4 tests was abnormal, only 10% of the subjects were responders.

The authors' definition of "physiologically relevant" response is questionable. Pathophysiological states may exist in sideropenia (see above). The response of laboratory tests identifying subclinical iron-deficiency may be very relevant but was not considered in the study.

Many of the 55% of patients termed "non-responders," who met the criteria of low Hgb and at least one other abnormal test, undoubtedly had an increase in SF or a decrease in FEP.

Cook et al. (6) measured SF, FEP, SAT, and Hgb in 1,564 subjects in the state of Washington. Iron-deficiency was defined as 2 or 3 abnormal values among SF, FEP, and SAT. The reason given was that in patients with 1 of 3 values abnormal the incidence of anemia as determined by low Hgb value was only 10.9%, vs. 8.3% for the entire population.

Cook et al. have assumed that the criteria for iron-deficiency is the predictability of various laboratory values for low Hgb values. Iron-deficiency, though, is well known to exist independently of anemia (47,81).

Using the authors' definition for iron deficiency, 9.3% of the population was iron-deficient and 4.2% of the population had IDA. 4.8% of adolescent girls had IDA while 10.6% had anemia. It is very unlikely that 55% of the adolescent girls who had anemia did not have iron-deficiency as the etiologic factor, since iron-deficiency is the major cause of anemia in adolescents (1).

Of the 1,564 subjects, 527, or 34%, had at least 1 of 3 abnormal tests among FEP, SAT, and SF. 441, or 28%, had abnormal FEP, SF, or FEP and SF. Since SAT is often not a reliable indicator of iron-deficiency (47), one might postulate that 28% is an accurate indication of the frequency of iron-deficiency in this population.

EPIDEMIOLOGICAL STUDIES OF IRON-DEFICIENCY IN ADOLESCENT FEMALES

Hillman and Smith (4) studied anemia in 5,597 subjects in Brooklyn, N.Y., from 1956 to 1965. Black, Puerto Rican, and white females from 10 to 14 years of age and from 15 years of age and older were considered anemic if the hemoglobin was lower than 11 g/dl. Among black females, 43% of 73 from 10 to 14 years and 40.1% of 237 15 years and over were anemic. Among Puerto Rican females, 27.3% of 121 from 10-14 years and 35.7% of 454 15 years and over were anemic. Among white females, 20.5% of 44 from 10-14 years and 20.1% of 169 15 years and over were anemic. Anemia was found to be greater among welfare recipient families than non-recipients in most racial and age groups. No statistical studies were performed.

Pearson et al. (2) studied 3,000 black subjects living in Gainesville, Florida, between 1964 and 1967. 726 were 10-14 years (males and females) and 220 were 14-17 year old non-pregnant females. Anemia was defined as Hct $<34\%$. Anemia was assumed to be due to iron deficiency based upon the hypochromic microcytosis of 36 infants with Hct $<30\%$ and the response to iron therapy of 193 4-6 year old children. 2.6% of the 12-14 year group and 8.0% of the 14-17 year female group were anemic. 23.9% of 213 14-17 year pregnant subjects were also found to be anemic. The authors concluded that the limited ability of adolescent females to respond to the increased iron needs of pregnancy indicated marginal reserves of iron in that age group.

Brown et al.(3) studied black junior high school students (12-15 years old) in Philadelphia. The children came from homes of IV and V in the Hollinghead Rates of socioeconomic background (105). Of the females without G-6-PD deficiency or sickle-cell disease, 117 of 769, or 15.2%

had Hgb <11.5 g/dl. 11.4% at 12 years, 13.2% at 13 years, 17.3% at 14 years, and 27.3% at 15 years were anemic. Anemia was assumed to be due to iron-deficiency due to an association with microcytosis and hypochromia.

Katzman et al.(98) studied 1,789 individuals at an inner-city HMO in New Haven, Connecticut, from 1968-70. 56% were black, 33% were Hispanic, and 11% were white. A Hgb of 12.0 g/dl and Hct of 35% were considered LNLs for 14-21 year old females. 32.7% of 150 females aged 14-21 years were considered anemic on the basis of low Hgb values. 13.8% of females aged 14-21 years had Hct <35%. 11.1% of 72 14-17 year old females had Hct <34% (vs. 8.0% in the Gainesville study). The anemia in this study was assumed to be due to iron-deficiency based upon study of the blood smears. The large discrepancies between the results based on Hct and Hgb suggest that the LNL used for Hgb was too high.

Katzman et al. also studied interracial differences in the prevalence of anemia. Mean Hgb and Hct values were lowest among black females in the group aged 14-21 years, followed by Hispanics, and then whites. The frequency of anemia in blacks was far higher than in Hispanics and whites based upon Hgb (44% vs. 20%) and Hct (20.7% vs. 0%).

In the preceding studies which compare IDA among racial groups, the prevalence may be overestimated among blacks due to potentially different LNLs for Hgb (100, 101, 102). In none of the studies was menstrual status determined, nor were patients questioned regarding past iron supplementation. Studies relying upon microcytosis and hypchromia as the criteria for iron-deficiency may not be accurate due to the high incidence of thalassemia trait among blacks (96, 97).

Information concerning sideropenia in large surveys is sparse, particularly among adolescents. Scott and Pritchard (78) performed bone marrow aspiration on 114 white female college students aged 19-25 years in Texas during the mid '60s. Stainable iron was totally absent in 24% of the subjects. Iron stores were assessed by quantitative phlebotomy in 4 subjects who were without bone marrow stainable iron. Iron stores averaged 52 mg, equivalent to 68 ml of blood. Two-thirds of the subjects were believed to have less than 350 mg storage iron.

Cook _et_ _al_. (6) studied 1,564 subjects in the state of Washington. The overall racial distribution could not be determined from the data presented, although 2% were black and 3% American Indian. 125 were females aged 12-17 years. Laboratory data for the 125 females were 24.5% <12 ng SF/ml, 5.3% >100 mg FEP/dl pRBC, 15.2% <15% SAT, and 10.6% <12 g Hgb/dl. Iron deficiency was defined as 2 or 3 abnormal values among FEP, SAT, and SF. 8.8% were considered iron-deficient. 4.8% were iron-deficient and anemic. Since false-low values for SF are seldom found (75,77), 8.8% is certainly a low estimate of iron-deficiency in this population.

PURPOSES AND HYPOTHESES

In the present study, the iron status of an inner-city population of adolescent girls was investigated. In the community studied, most of the subjects were of black and Hispanic heritage, and of a homogeneous socioeconomic status. Data were obtained by questionnaire, chart review, and phlebotomy.

The incidence of IDA in the study group, as determined by low Hgb and MCV values, was expected to be similar to that obtained by other authors on similar populations. It was also expected that IDA, as determined by abnormal SF, FEP, and Hgb values, would be equivalent to IDA as determined by low Hgb and MCV values.

The major hypothesis of the study was that the incidence of iron-deficiency indicated by abnormal SF and FEP values alone would be significantly greater than that discovered with the use of Hgb and MCV values alone; in addition to the iron-deficiency anemia detected by traditional tests (MCV and Hgb), a significant number of subjects would have subclinical iron-deficiency (iron-deficiency in the absence of anemia). Because of the high risk of IDA among adolescent girls, a significant number of borderline cases were expected to be sideropenic.

Other objectives included determining the value of using SF, FEP, Hgb, and MCV measurements, alone or in combinations, for the detection of iron-deficiency and anemia. Comparisons of results were made between the 2 major racial groups, both within the study and with past studies.

MATERIALS AND METHODS

Subjects were drawn from the Yale-New Haven Hospital (YNHH) Adolescent Clinic and the Hill Health Center, both in New Haven, Connecticut. The medical costs of the majority of the patients attending the two clinics are paid by public agencies. Most live in the inner-city districts of New Haven.

The study was approved by the Human Investigations Committee of YNHH and Yale Medical School. Permission to include each subject in the study was obtained orally from a patient, or in writing if the patient attended clinic alone(see Appendix A).

Most of the subjects were attending clinic for routine physical exams. A chart review was performed on each. Patients with evidence of blood loss, renal disease, inflammatory disease, lead poisoning, or chronic disease were eliminated from the study. The iron status of 76 patients, aged 12 to 18, was investigated.

Each subject completed a questionnaire in which information was requested regarding age, race, menses, pregnancy, use of iron supplements, and major medical problems (see Appendix B). Two tubes of blood, one coagulated and one EDTA anti-coagulated, were obtained from each subject. The serum from the coagulated tubes was frozen and stored. The anticoagulated samples were stored in the dark at 4° C.

Electrophoresis was performed on all samples within 1 week on a cellulose acetate plate obtained from Helena Laboratories of Beaumont, Texas.

The serum ferritin values were determined by radioimmunoassay using the "Fer-Iron" kit obtained from Ramco Laboratories Inc. of Houston, Texas. The method is based upon that of Miles _et al_. (106). The tests

were performed in the clinical laboratories of YNHH, where values <12 ng/ml are considered abnormal, and values of 12-14 ng/ml are considered "suspicious."

ZPP values were obtained using a hematofluorometer produced by AVIV Associates, model ZPP, using a method based upon that of Blumberg et al. (86). Anti-coagulated samples were analyzed within 1 week after storage at 4 ° C. in the dark. In the YNHH laboratory, a "normal" range is considered to be 9-22 μg/dl blood, as determined by eliminating the high and low 5% of values from samples obtained from medical student volunteers.

Hgb values were measured with a hemoglobinometer, model number HGBRZ, produced by Coulter Electronics. Refrigerated samples were analyzed within 24 hours, while fresh samples were tested within 4 hours.

MCV values were obtained by electronic counting with a Coulter counter, model number FN. Storage was identical to that of the Hgb samples.

In all tests of statistical significance, the student's t-test was employed.

RESULTS

QUESTIONNAIRE RESULTS

Of the 76 subjects studied, 6 were premenarchal and 5 were using iron supplements. The group without menses included 3 blacks, 2 Hispanics, and 1 white (Table 1). The age range was 12-14 years, with a mean of 13 years. The mean SF was 25 ng/ml, the mean ZPP 16 μg/dl, the mean Hgb 12.6 g/dl, and the mean MCV 83 fl. One subject had an MCV<LLN, none had a Hgb >11.5 g/dl, and one had a SF <18 ng/ml. Among the iron supplement users were 2 blacks and 3 Hispanics, all of whom were postmenarchal. They ranged in age from 13 to 18 years, with a mean of 15 years. Their mean SF was 37 ng/ml, the mean ZPP 20 μg/dl, the mean Hgb 13.2 g/dl, and the MCV 88 fl. One of the five had a ZPP ≥30 μg/dl.

The age ranged from 12 to 18 years, with a mean of 14.7 years for the entire study group (Figure 1). After elimination of the 11 subjects discussed above, the mean was 14.8 years.

Of the 76 subjects, 52 (68%) were black, 21 (28%) Hispanic, and 3 (4%) white. Of the 65 girls with menses and without oral supplementation ("subgroup") 47 (72%) were black, 17 (26%) Hispanic, and 1 (2%) white.

None of the patients were known to be pregnant. Appropriate laboratory tests performed in doubtful cases were negative.

LABORATORY RESULTS

As noted above, SF values <12 ng/ml are certainly abnormal, while values ≥12 ng/ml and <18 ng/ml are probably indicative of absent bone marrow iron. Of the 76 subjects, 13 (17%) had SF <12 ng/ml and 23 (30%) <18 ng/ml(Figure 2). Of the subgroup of 65, 13 (20%) had a SF <12 ng/ml and 22 (34%) <18 ng/ml. Among the 65, the mean SF was 32 ng/ml while the median was 24 ng/ml. Of the 9 subjects with Hgb <11.5 g/dl, 5 had a SF <12 ng/ml while 8 had SF <18 ng/ml.

Table 1

Characteristics of premenarchal and iron supplement-using groups

	Pre-menarchal girls	Girls using iron supplement
Total number	6	5
Number Hispanic	1	3
Number black	3	2
Number white	2	0
μ age (yr)	13	15
μ SF (ng/ml)	25	37
μ ZPP (μg/dl)	16	20
μ Hgb (g/dl)	12.6	13.2
μ MCV (fl)	83	88

μ = mean.

Age distribution of all subjects

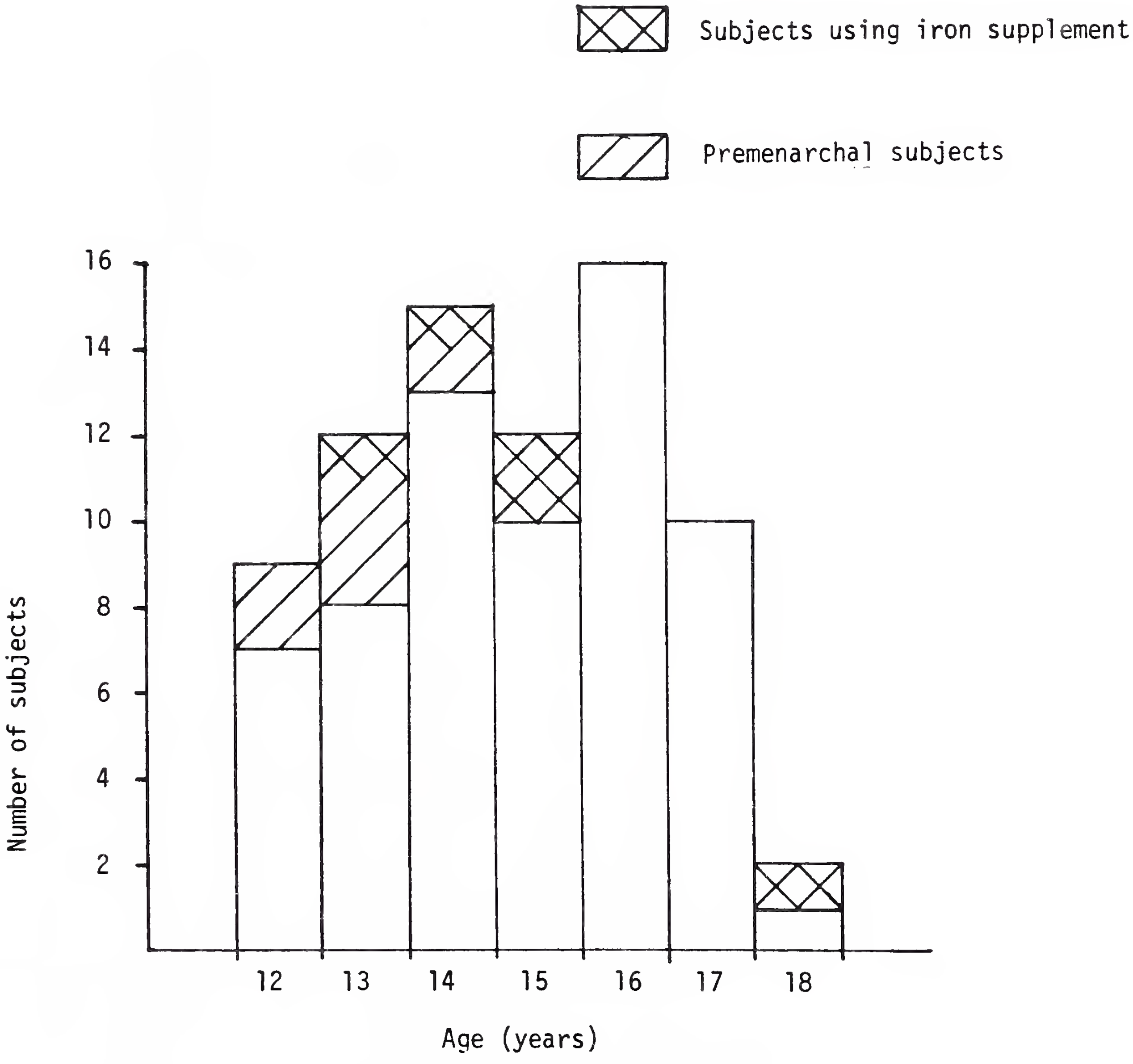


Figure 1

Distribution of serum ferritin values

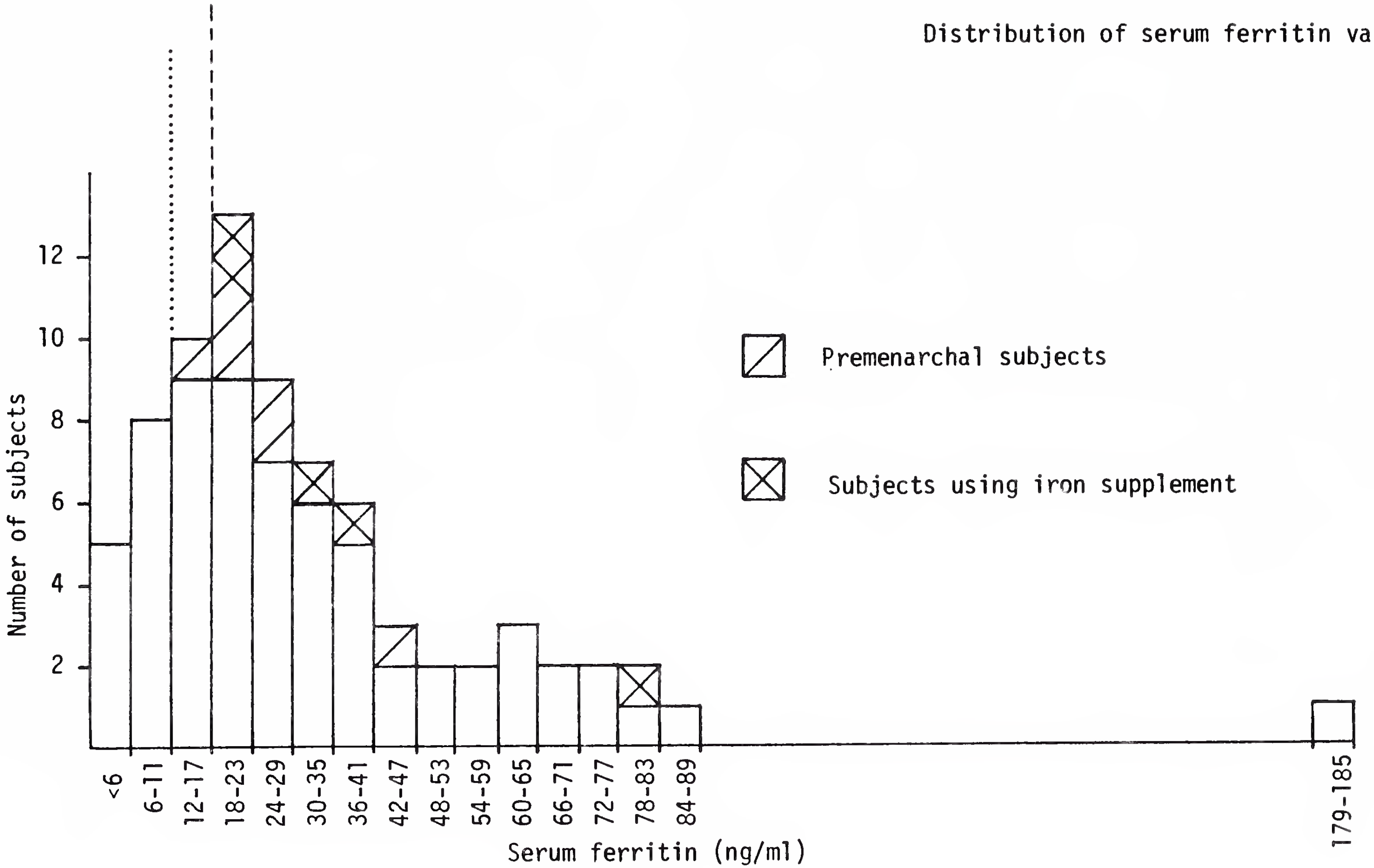


Figure 2

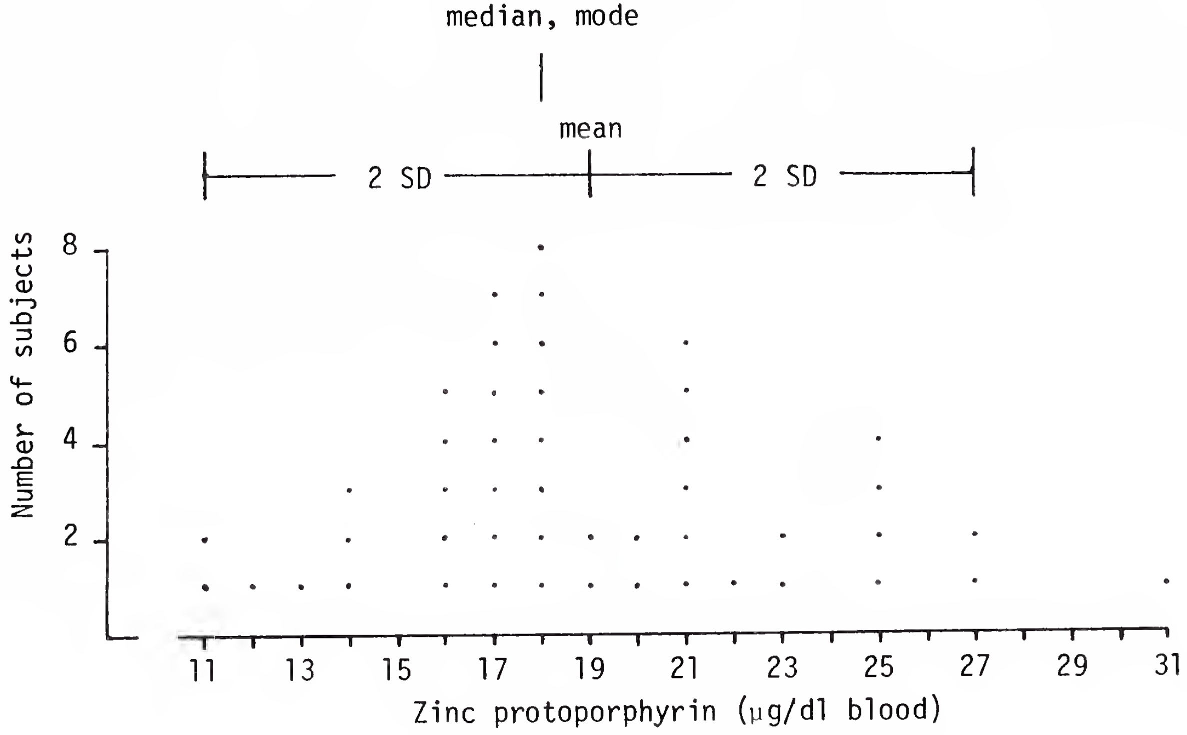
ZPP values were analyzed among patients without evidence of iron-deficiency (MCV >77 fl, Hgb ≥ 11.5 g/dl, SF ≥ 18 ng/ml). As shown in Figure 3, the mean was 18 and the mode and median were 19 μg/dl. The standard deviation was calculated as 4 μg/dl. Since the median, mode, and mean were roughly equivalent, a 95% confidence limit of 11-27 μg/dl was estimated. Using the method of the YNHH clinical laboratory (90% confidence, see above), the normal range was estimated as 12-27 μg/dl. A ZPP <30 μg/dl was considered within normal limits in the present study.

The ZPP values of the group of subjects with MCV >77 fl, Hgb ≥ 11.5 g/dl, and SF ≥ 12 and <18 ng/ml were compared to those of the aforementioned group with SF ≥ 18 ng/ml, and determined to be significantly ($P < 0.001$) different. The mean ZPP of the group with intermediate SF was 30 μg/dl.

Ten (13%) of the 76 subjects had abnormal ZPP values (Figure 4). Nine (14%) of the subgroup of 65 had ZPP ≥ 30 g/dl. The 65 subjects had a mean value of 23 μg/dl. Four of the 9 subjects with Hgb <11.5 g/dl had ZPP ≥ 30 g/dl.

As previously mentioned, several different values have been used for the LNL of Hgb. Dallman and Siimes (94) found that the 95% confidence limit for the Hgb of white adolescent girls was 12.0 g/dl. Evidence exists that the LNL for the black population should be 0.5 g/dl lower (100, 101, 102); a LNL of 11.5 g/dl was used in a study of black females by Brown _et al_. (3). Brown _et al_. presented data that showed significant differences between mean MCV values among subjects with Hgb <11.5 g/dl and iron-replete subjects with Hgb >14.0 g/dl and <14.9 g/dl, suggesting that 11.5 g/dl was a valid LNL. A similar analysis was performed

Distribution of zinc protoporphyrin values in subjects without evidence of iron-deficiency[1]



[1] SF $\geq$18 ng/ml, Hgb $\geq$11.5 g/dl, MCV >77 fl.

Figure 3

Distribution of zinc protoporphyrin values

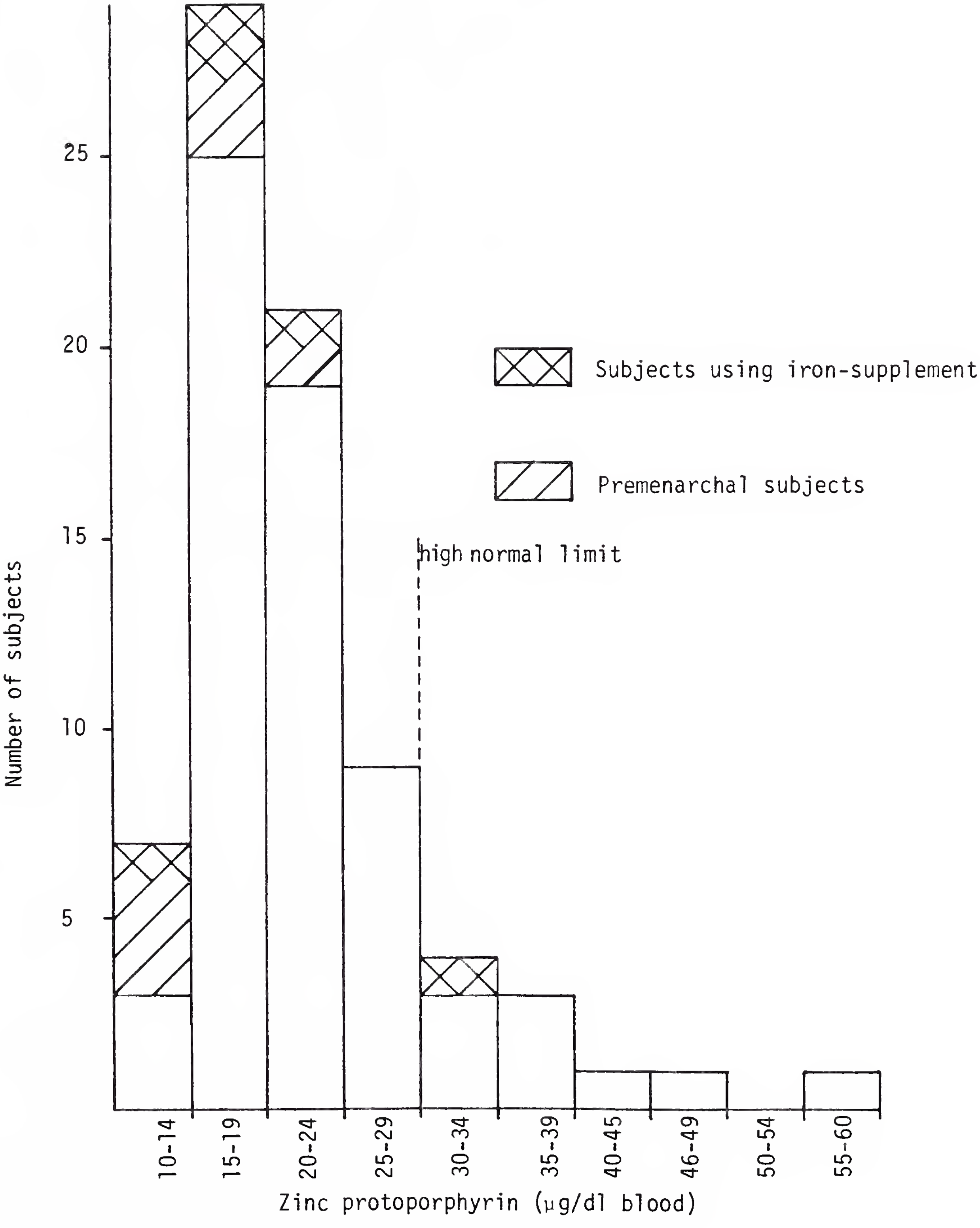


Figure 4

in the present study (see Table 2). Mean SF, ZPP, and MCV were compared for the 76 subjects in four Hgb ranges: 1) 10.0-11.4 g/dl, 2) 11.5-11.9 g/dl, 3) 11.5-12.4 g/dl, and 4) 13.0-14.4 g/dl. Group 4 was similar to the group of subjects receiving iron supplements (see Table 2). The mean MCV for groups 1 and 4 were significantly different ($P < 0.001$), but not for group 1 versus groups 2 and 3. Groups 2 and 4 were also significantly different ($P < 0.01$). There were no significant differences among any of the groups for ZPP values, although group 1 had a higher mean than the other 3 groups. SF values were significantly different between groups 1, and 2, 3 and 4 ($P < 0.01$, 0.01, and 0.05 respectively). SF for groups 2 and 4 were not significantly different ($P > 0.6$). 25% of the patients in group 2 had evidence of iron-deficiency (abnormal SF &/or ZPP), while 89% of the patients in group 1 had such evidence.

The racial heterogeneity of the Hispanic population in the study does not allow a label of black or white for purposes of LNL Hgb. The mean values for the Hispanic and black subjects without evidence of iron deficiency (SF > 18 ng/ml, ZPP ≥ 30 μg/dl, and MCV > 77 fl) were found to be 12.9 and 12.8 respectively, a statistically insignificant difference (see Table 4). A Hgb LNL of 11.5 g/dl was used for black and Hispanic girls, while a LNL of 12.0 g/dl was applied to white subjects.

The distribution of Hgb values is presented in Figure 5. Seven black subjects, 2 Hispanics, and no white subjects were anemic. Nine of the total of 76 subjects (12%) and 9 of the subgroup of 65 subjects (14%) had Hgb < 11.5 g/dl.

As noted earlier (94), the 95% confidence limits for girls aged 12-13 years has been calculated at 77 fl, and 78 fl for girls aged 14-18 years. The distribution of MCV values is presented in Figure 6. Ten subjects, all black, had MCV < 77 fl (there were no subjects with an MCV of

Table 2

Comparison by Hgb range of SF, ZPP, and MCV mean values. The mean value for each laboratory test in the iron supplement-using group is also compared. (See text for statistical significance.)

Group	Hgb range	μSF (ng/ml)	μZPP (μg/dl)	μMCV (fl)
1	10.0-11.4	11	24	78
2	11.5-11.9	35	20	80
3	11.5-12.4	31	21	83
4.	13.0-14.4	42	21	89
iron-supplements	12.3-14.2 (13.2)[a]	37	20	88

[a] Figure in parentheses: the mean.

Distribution of hemoglobin values

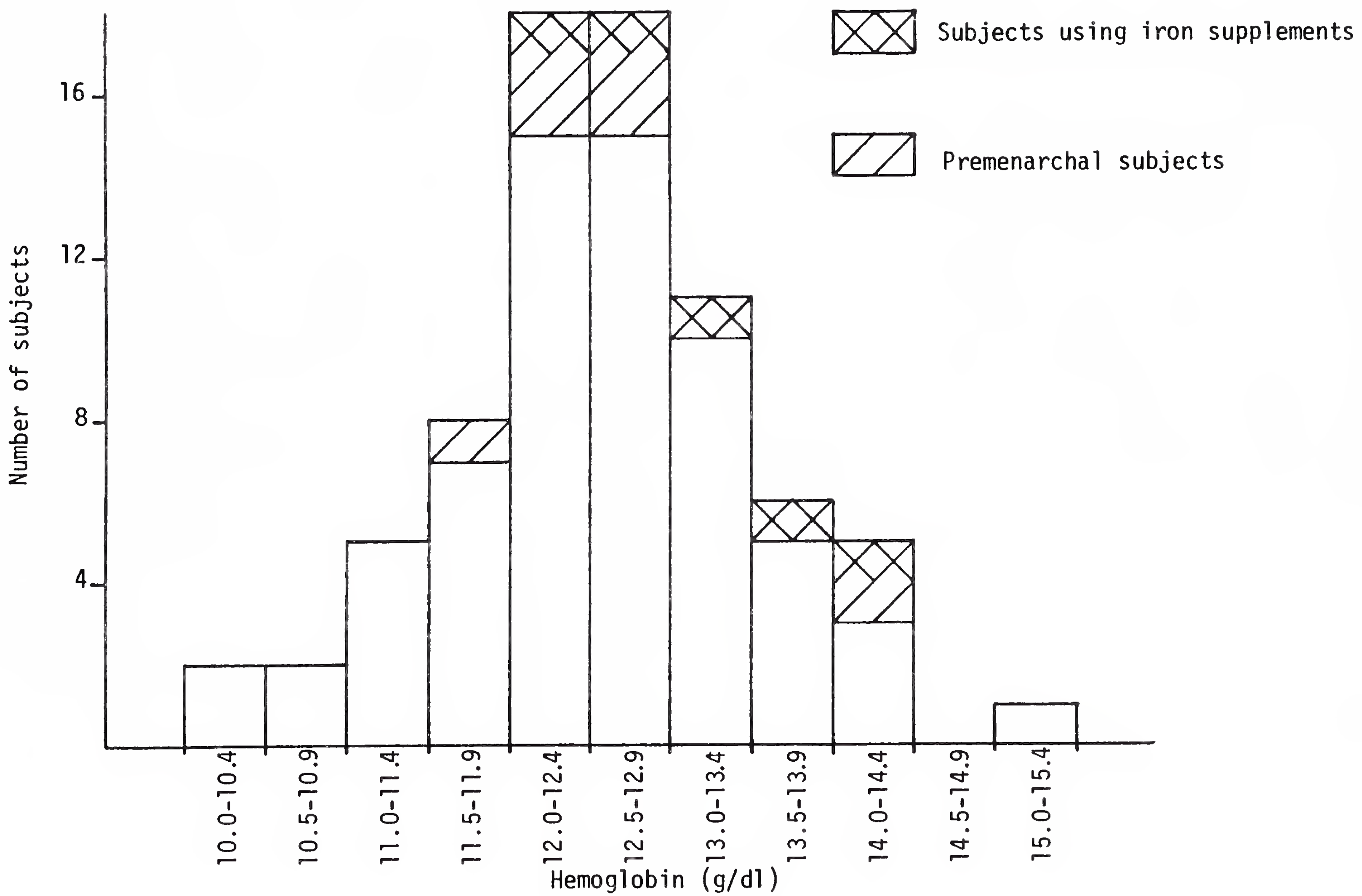


Figure 5

Distribution of mean corpuscular volume values

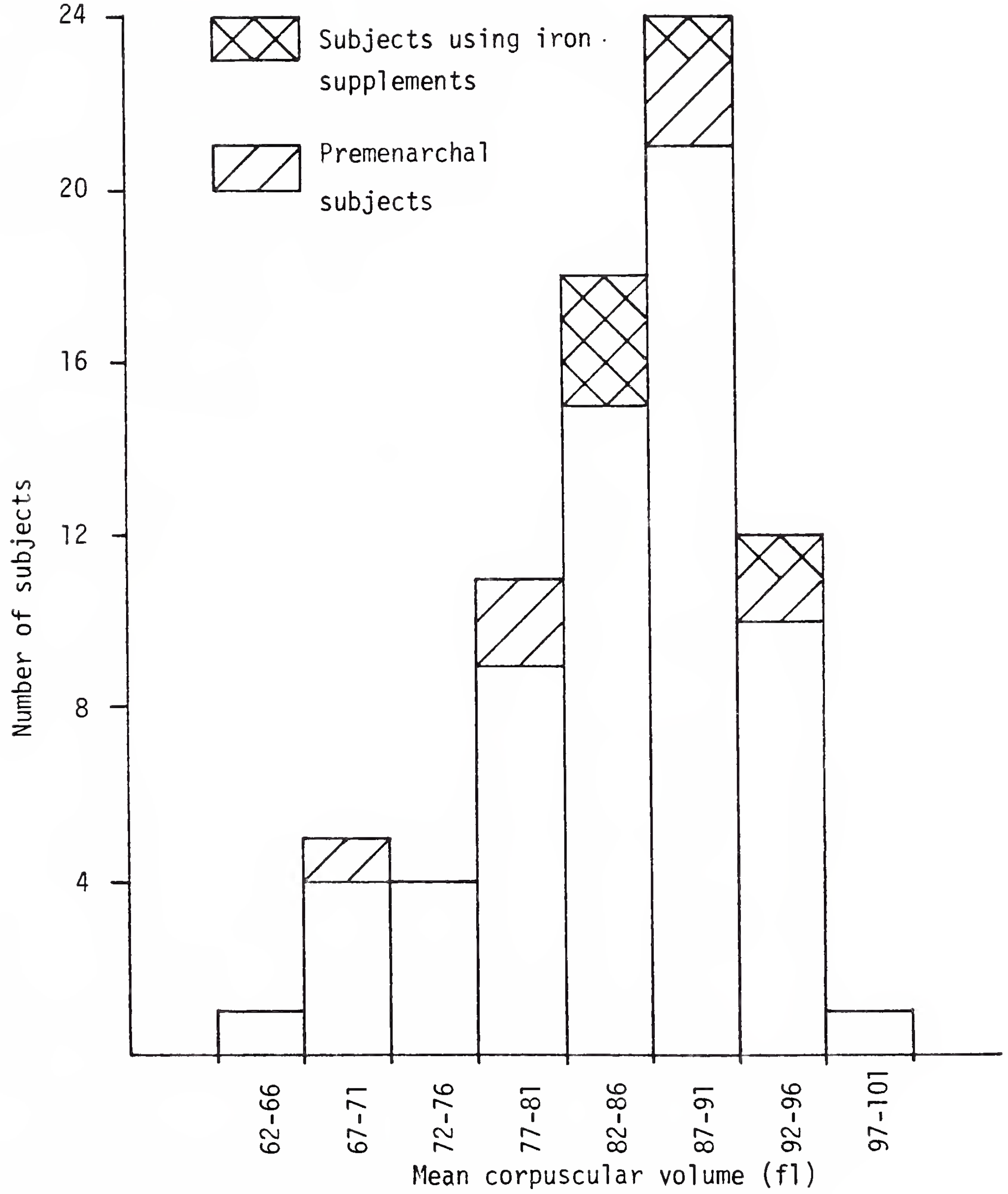


Figure 6

77 fl). Five had Hgb <11.5, all of whom had other evidence of iron-deficiency (SF<18 ng/ml, and/or ZPP $\geq$30 µg/dl). One had a Hgb of 12.0 g/dl, a ZPP of 41 µg/dl, a SF of 32 ng/ml, and an MCV of 64 fl. After 1 month of iron therapy, the subject had a Hgb of 13.3 and an MCV of 73 fl. Four subjects with abnormal MCV had no evidence of iron-deficiency, at least one of whom was found to be refractory to iron therapy. Among the 76 subjects, 6 of 10 had evidence of iron deficiency. Among the subgroup of 65, 6 of 9 had evidence of iron-deficiency.

Electrophoresis revealed no major hemoglobinopathies among the study subjects. Sixty-eight had hemoglobin AA, 4 hemoglobin AS, 2 hemoglobin AF, 1 hemoglobin AC., and 1 hemoglobin A? (? = chain with increased mobility. All laboratory iron studies were within normal limits for this subject). Quantitative electrophoresis for Hgb A_2 was negative for 1 subject with low MCV and no evidence for iron-deficiency. The others were not tested.

Subjects were analyzed utilizing 2 definitions of iron-deficiency. Criterion one for iron deficiency (ID_1) was a SF <18 ng/ml or ZPP $\geq$30 g/dl or both. Criterion two (ID_2) was SF <12 ng/ml or ZPP $\geq$30 ng/dl or both. IDA was defined as Hgb< 11.5 g/dl plus iron-deficiency as defined above. Twenty-three (35%) of the 65 subjects with menses and not employing iron supplements met ID_1 (see Figure 7). Eight had both abnormal SF and ZPP, 1 abnormal ZPP only, and 14 abnormal SF only. Eight had Hgb< 11.5, yielding 15 (23%) with subclinical iron deficiency. Of the total of 76 subjects, 25 (33%) met ID_1. Seventeen (26%) of 65 subjects met ID_2. Both SF and ZPP were abnormal in 6, ZPP only in 3, and SF only in 8. Six of 17 had Hgb<11.5, leaving 11 (17%) with subclinical iron-deficiency. Nineteen (25%) of 76 girls met ID_2. Two subjects qualifying for IDA employing ID_1 did not qualify using ID_2, although both had MCV suggesting iron-defi-

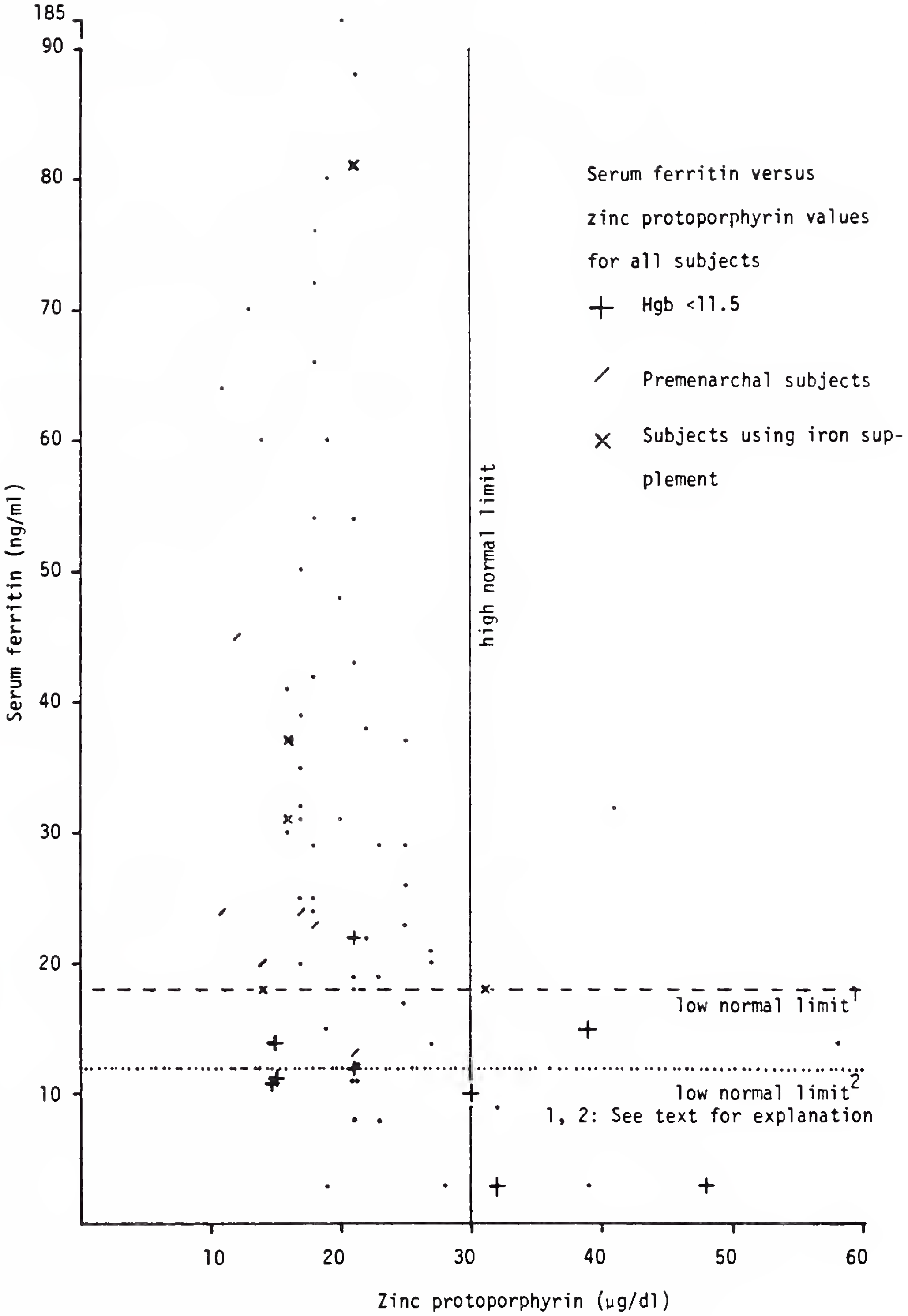

185
90
80
70
60
50
40
30
20
10
Serum ferritin (ng/ml)
10
20
30
40
50
60
Zinc protoporphyrin (μg/dl)
high normal limit
low normal limit¹
low normal limit²
1, 2: See text for explanation
Serum ferritin versus
zinc protoporphyrin values
for all subjects
+ Hgb <11.5
/ Premenarchal subjects
× Subjects using iron supplement

Figure 7

ciency (MCV of 69 and 70). Therefore, 8 subjects were considered to have IDA, translating to 11% of 76 subjects and 12% of 65 subjects. One subject had a Hgb <11.5 but did not meet ID_1 or ID_2.

Table 3 shows the percentage of subjects within various Hgb ranges with evidence of iron-deficiency. 89% of girls with Hgb <11.5 g/dl met ID_1, while only 67% met ID_2. In the Hgb range 11.5-12.4 g/dl, the percentages were 35% and 19% respectively. In the subjects with higher Hgb values, the percent iron-deficient was essentially constant regardless of the criteria employed. The laboratory values of the 47 black and 17 Hispanic postmenarchal girls free of iron supplements were compared (Table 4). Differences in mean values for age, SF, and ZPP were without significance. The mean MCV for black girls (84fl) was significantly less (P <0.02) than that for Hispanic girls (89fl). The mean Hgb for Hispanic girls was 0.4 g/dl greater than that for black girls but not statistically significantly different. In subjects without other evidence of iron-deficiency (SF ≥18 ng/ml, ZPP <30 µg/dl, and MCV >77 fl), black and Hispanic girls had Hgb of 12.8 g/dl and 12.9 g/dl respectively. In subjects with normal Hgb, SF, and ZPP values, mean MCV values were insignificantly different between the 2 racial groups. Using ID_1, 47% of Hispanic girls and 32% of black girls were iron-deficient. Employing ID_2, 41% of Hispanic girls and 21% of black girls were iron-deficient. Only 6% of the Hispanic subjects had IDA, as opposed to 15% of the black subjects.

Table 3

Comparison by Hgb range of the percent of subjects meeting criterion 1 (SF <18 ng/ml &/or ZPP ≥ 30 μg/dl) and criterion 2 (SF <12 ng/ml &/or ZPP ≥ 30 μg/dl) for iron-deficiency

Hgb range	% of subjects, ID_1	% of subjects, ID_2
10.0-11.4	89	67
11.5-12.4	35	19
12.5-13.4	21	17
$\geq$13.5	17	17

Table 4

Comparison by race of age, hematological test results, and statistical significance of differences in values

μ values	Hispanic girls	black girls	probability
Age (yr)	15.1	14.6	>0.2
SF (ng/ml)	33 (24^c)	31	>0.8
ZPP (μg/dl)	25	22	>0.1
Hgb (g/dl)	12.7	12.3	>0.1
Hgb^a (g/dl)	12.9	12.8	>0.6
MCV (fl)	89	84	<0.02
MCV^b (fl)	89	86	>0.2
% of subjects			
ID_1	47	32	
ID_2	41	21	
IDA	6	15	

[a] Without other evidence of iron-deficiency (SF ≥18 ng/ml, ZPP <30 μg/dl, MCV >LNL).

[b] Without other evidence of iron-deficiency (SF ≥18 ng/ml, ZPP <30 g/dl, Hgb ≥11.5 g/dl).

[c] Figure in parentheses is the mean if the single distorting value of 185 ng/ml is eliminated (see text for explanation).

ID_1 - Iron-deficiency criterion #1 (SF <18 ng/ml &/or ZPP ≥30 μg/dl).

ID_2 - Iron-deficiency criterion #2 (SF <12 ng/ml &/or ZPP ≥30 μg/dl).

IDA - Iron-deficiency anemia (meeting criteria of Hgb<11.5 g/dl and ID_1).

DISCUSSION

The population studied, of uniformly low socioeconomic status, would be expected to yield especially high rates of IDA. The high incidence of IDA demonstrated in past studies of adolescent girls was confirmed in the present study.

Past studies have failed to differentiate between menarchal and pre-menarchal girls, as well as between those ingesting and not using iron supplements. Elimination of the two groups, however, failed to create any statistically significant changes in the data.

The incidence of iron-deficiency determined in any population study depends on the laboratory criteria employed. The previously discussed literature suggests that SF <12 ng/ml is universally accepted as abnormal. The intermediate zone ≥ 12 ng/ml and <18 ng/ml has been associated with absent bone marrow stores of iron, and may be regarded as a "suspicious" zone. Since SF can quickly rise with ingestion of iron, 12-17 ng/ml is a borderline range, at the least; a subject with SF of 14 may have had a SF of 9 two days earlier. The use of a LNL of 18 ng/ml was suggested to be more legitimate by intrastudy observations. Subjects in the SF range of 12-17 ng/ml had significantly greater ZPP values when compared to subjects with SF ≥ 18 ng/ml. The percent of iron-deficient subjects among subjects with Hgb >12.4 g/dl was the same using ID_1 and ID_2 (Table 3), suggesting that subjects who are comparatively iron replete are just as unlikely to have SF <18 ng/ml as SF <12 ng/ml. Since high false SF values are much more common than low false values (as previously discussed)(75, 77, 79),

the use of SF as a criterion for iron-deficiency is unlikely to result in an overestimate.

As discussed above, ZPP values have not yet been standardized among different laboratories (87). A value higher than the 95% confidence limit and utilized by previous studies was defined as the HNL. Although the correlation between low Hgb and high ZPP was poor (4 high ZPP among the anemic subjects), most high ZPP values were associated with low SF (Figure 7). In the only case where a high ZPP value was associated with a high SF, the MCV and Hgb were found to respond dramatically with iron supplementation.

The statistical significance of the differences in SF between subjects with Hgb <11.5 g/dl and all other groups suggests a true difference in iron status. A statistically significant microcytosis occurs not only in subjects with Hgb <11.5 g/dl, but also in the 11.5-12.4 g/dl range. The percent of subjects with abnormal SF and ZPP is much higher with Hgb <11.5 g/dl than in other groups using ID_1 or ID_2 (Table 3). Employing ID_2, there is no difference between the Hgb ranges 11.5-12.4 g/dl, 12.5-13.4 g/dl, and >13.5 g/dl. Employing ID_1, the 11.5-12.4 g/dl range is intermediate in percent of iron-deficiency. The significant microcytosis in the 11.5-12.4 g/dl group suggests that there is a true difference in iron status, in turn suggesting that ID_1 is more valid than ID_2.

A single Hgb value cannot be used to define anemia in individual cases (47). For the purposes of population studies, a value must be found which has approximately equal false-positives and false-negatives. One of the subjects with Hgb $<11.g$ g/dl had no evidence of iron-deficiency (SF of 22 ng/ml, ZPP of 21 μg/dl). One or more subjects with Hgb ≥ 11.5 g/dl may have been anemic. A large number of

"non-anemic" subjects had abnormal SF, ZPP, and MCV, and at least one responded to iron therapy with an increase in Hgb from 12.0 to 13.3 g/dl. The number of anemic individuals may have been underestimated.

Four of the 10 MCV <77 fl were not accompanied by other evidence of iron-deficiency. One of the four was shown to have normal amounts of Hgb A_2. Thalassemia-trait is the most likely etiology for the low MCV values. 8% (of 52 black girls) is higher than the expected prevalence of thalassemia-trait resulting in decreased MCV (β-thalassemia trait 0.5-1.0%, α-thalassemia trait 1.9% (96)). Utilization of iron therapy and subsequent follow-up might reveal one or more cases of significant response in Hgb and MCV values, suggesting undetected IDA. One subject was resistant to iron therapy. Follow-up was unavailable in the remaining 3 girls.

Iron-deficiency was present in 25% to 35% of subjects, depending on the criteria used. Subclinical iron-deficiency was present in 17% to 23% of the population surveyed. The physiological significance of this large percentage is uncertain, as noted above. Only 8% had ZPP $\geq$ 30 μg/dl independently of anemia. As previously discussed, limitation of heme production, reflected by high ZPP, is more likely to be associated with limitation of iron-dependent enzyme production than low SF alone. One may safely conclude, though, that a large and unacceptable number of girls are at risk for the known and postulated pathological consequences of IDA.

The small sample of Hispanic girls limits the validity of the comparison with the black subjects. Iron-deficiency appears to be greater in the Hispanics, while IDA is greater among the blacks. This difference may be due to a need for a higher Hgb value for defining anemia among

Hispanics, although increasing the cut-off to 12.0 g/dl would not increase the percentage of IDA. It may also be due to a greater susceptability among blacks to a decrease in Hgb with iron-deficiency. Such an explanation could also explain the previously noted difference in hemoglobin values between black and white populations (101,102,103). In the present study, no significant difference was found in mean Hgb of iron-replete black and Hispanic girls (Table 4).

Katz et al. (98) studied the same New Haven community in the early 1970's. A Hgb LNL of 12.0 g/dl was employed. 44% of 77 black females aged 14-21 years were found to be anemic. 6.2% of all subjects (Hispanic and black) aged 10-14 years were anemic. In the present study, 27% of all 52 black girls had Hgb <12.0 g/dl. 23% of 35 girls aged 14-18 years and 35% of 17 girls aged 12-13 years had Hgb <12.0 g/dl. The mean Hgb of females in the Katz study was 12.6 g/dl versus 12.4 g/dl in the present study, suggesting a smaller statistical deviation in Hgb distribution in this study. 20.6% of 63 Hispanic females aged 14-21 years had Hgb <12.0 g/dl in the earlier study. 14% of the 21 Hispanic girls in this study had Hgb <12.0 g/dl.

Brown et al. (3) studied 769 black girls aged 12-15 years in inner-city Philadelphia. 15.2% had Hgb <11.5 g/dl, with an overall mean of 12.5 g/dl. 9% of 34 black girls aged 12-15 years were anemic in the present study, with an overall mean of 12.4 g/dl. 13% of 52 black girls aged 12-18 were anemic, with an overall mean of 12.4 g/dl.

The estimated percentage of anemia in the present study is slightly lower than that of past studies. This may be due to improved nutrition over time, different subject populations, or inadequate sample size in the present study.

Cook et al.(6) used a more stringent definition for iron-deficiency, as previously noted. 8.8% of girls 12-17 years were found to be iron-deficient, with 4.8% IDA. 24.5% had Hgb<12 g/dl, versus 17% in the present study. 5.3% had high FEP values, versus 13% in the present study. Using ID_2, the percent of adolescent girls with iron-deficiency in the Cook study would be similar to that in the present study (25%). Cook, though, had a lower percent of low Hgb values (10.6% < 12.0 g/dl). The high number of abnormal SF values in Cook's study, compared to the present study, may to the present study, may be due to the use of a different assay.

The present study is limited by small sample size, as well as by the unavailability of follow-up data after implementation of iron therapy. The population seen in the clinics is likely to be a sample skewed from the normal population. They are probably more likely to have had adequate past medical care and more likely to have a present illness (although patients with known disease were excluded).

CONCLUSIONS

The inner-city female adolescent population has a high incidence of iron-deficiency, less than half of whom are identified by low Hgb and MCV values. The clinical significance of the subclinical iron-deficiency state is unknown, although a few studies have suggested pathological consequences of sideropenia associated with high FEP values. Analysis of the data in this study suggests that SF values between 12 and 18 ng/ml may be evidence of significant iron depletion. It is suggested that all girls with Hgb values <11.5 g/dl receive iron supplements. Girls with Hgb between 11.5 and 12.4 g/dl should be considered for iron therapy due to the large percentage of suspected iron-deficiency present in this range.

APPENDIX A

You are invited to participate in a study of iron nutrition in teenage girls living in New Haven. Many girls of your age do not get enough iron in their diets. We are trying to find out how common this lack of iron is in the patients we see here. The study involves one needle stick to obtain two small tubes of blood, and the completion of a short questionnaire. If it is found that you are not getting enough iron, your physician will be notified, and may give you a prescription for iron pills. In the study itself, you will be represented by a number and your name will not be used in any report. Your results will be entered in your clinic chart for use by your doctor. We shall not tell the results to anyone else without your permission. You are free to decide whether or not to participate, and your decision will have no effect on your future relationship with the clinic. If anything is unclear, feel free to ask questions before deciding.

Authorization: I have read the above and decide that..................................
(name of subject)
will participate in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

....
Signature

....
Relationship (self, parent, guardian, etc.)

....
Date

....
Signature of Principal investigator Telephone
or

....
Signature of Person obtaining consent Telephone

APPENDIX B

IRON NUTRITION QUESTIONNAIRE

1. How old are you? ____

2. What is your race?

   Black ☐ White ☐ Hispanic ☐ Other ☐

3. A. Do you have your menstrual period yet?

   Yes ☐ No ☐

   B. If yes, how old were you when you first got it? ____

   C. If yes, do you get your period every month?

   Yes ☐ No ☐

   D. Have you ever been pregnant? Yes ☐ No ☐

   E. Are you pregnant now? Yes ☐ No ☐

4. Do you take iron pills or vitamins with iron?

   Yes ☐ No ☐

5. Do you have any important medical problems?

   Yes ☐ No ☐

   If so, what kind? ________________________________

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